ALTERNATIVE SOURCES OF OMEGA-3 OILS FOR BARRAMUNDI, *Lates calcarifer*, AQUACULTURE

By

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A thesis submitted in fulfilment of the requirements for the degree of

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National Centre for Marine Conservation and Resource Sustainability

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STATEMENT OF CO-AUTHORSHIP

The following people contributed to the publication of the work undertaken and included in the thesis body, which is:

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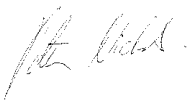
Supervisor	Dr. P. D. Nichols	 .....
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ABSTRACT

Fish oil (FO) is the major source of dietary lipid in carnivorous fish feeds including barramundi, *Lates calcarifer*, which is widely farmed in Asia and Australia. However, recent increases in FO prices, increased demand and the foreseen inability of wild fisheries to meet future requirements have created a need for cheaper and more sustainable alternatives. Vegetable oils (VO) can be produced in sufficient quantities to meet the growing aquaculture demand, although they lack the long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) beneficial to human consumers. Some VO like rapeseed oil (RO), echium oil from *Echium plantagineum* (EO) and linseed oil (LO) have high levels of n-3 and n-6 short-chain ($\leq C_{18}$) PUFA that can accumulate or be converted into LC-PUFA by some fish species, although generally at low efficiency, and not to docosahexaenoic acid. In a series of comparative and factorial experiments, I investigated the growth and lipid changes of barramundi fed different dietary oils: FO, RO, LO and EO over conditions covering: a range of salinities and temperatures, subject to immunity stress or supplemented with plant-derived bioactive ingredients. In general, growth performance parameters were comparable for FO and VO treatments, and resulted in accumulation of VO-derived n-3 and n-6 PUFA. Salinity has no direct effect on growth or lipid metabolism regardless of the dietary lipid source. Endogenous conversion by barramundi of dietary PUFA into LC-PUFA is limited by more than one rate-limiting step and there is a preference for incorporation of LC-PUFA into the polar lipid fraction rather than neutral lipid. The growth of barramundi slowed at sub-optimal (20°C) temperature compared to optimal (30°C) temperature. PUFA from dietary VO deposits in muscle and are maintained under rapid temperature decreases. In contrast, excess LC-PUFA from FO depleted faster than occurs in VO fed fish. The production of pro-inflammatory eicosanoids in fish fed FO was lower than for fish fed VO following bacterial infection. EO significantly suppressed the production of the pro-inflammatory mediators compared to RO. Sesamin, a lignan in sesame seed, enhanced the conversion of dietary PUFA into LC-PUFA for the n-3 series rather than n-6 in early juvenile barramundi. However, sesamin had negative impact on fish growth at this early life-stage. Barramundi fed on VO are a rich source of LC-PUFA precursors, α-linolenic and stearidonic acid, and grow well under the different environmental conditions that are typical of outdoor barramundi farms. The use of terrestrial VO containing the LC-PUFA precursors and plant-derived bioactive compounds show promise for use in barramundi aquafeed in terms of fish growth and health as either partial or complete alternatives for FO. However, using currently available VO, high content of the n -3 LC-PUFA is not achieved.

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"*If I have seen further, it is by standing on the shoulders of giants*."

Ramez

LIST OF ABBREVIATIONS

The following abbreviations are frequently used in this thesis:

AD, apparent digestibility
ALA, α-linolenic acid
ANOVA, analysis of variance
ARA, arachidonic acid
CMC, carboxymethyl cellulose
DHA, docosahexaenoic acid
DM, dry matter
DNA, deoxyribose nucleic acid
DPA, docosapentaenoic acid
EDTA, ethylenediaminetetraacetic acid
EFA, essential fatty acids
EO, *Echium* oil
ETA, eicosatetraenoic acid
FA, fatty acids
FAMB, fatty acid mass balance
FAME, fatty acids methyl esters
FC, total feed consumption
FER, feed efficiency ratio
FFA, free fatty acids
FID, flame ionisation detection
FM, fishmeal
FO, fish oil
GC, gas chromatography
GC-MS, gas chromatography mass spectrometry
GLA, γ-linolenic acid
LA, linoleic acid
LC-PUFA, long-chain ($\geq C_{20}$) polyunsaturated fatty acids
LO: linseed oil
mRNA, messenger ribonucleic acid
MUFA, monounsaturated fatty acids

PL, polar lipids

qRT-PCR, quantitative real time-polymerase chain reaction

RNA, ribose nucleic acid

RO, rapeseed oil

SDA, stearidonic acid

SEM, standard error of the mean

SFA, saturated fatty acids

SGR, specific growth rate

TAG, triacylglycerols

TLC, thin layer chromatography

TLE, total lipid extract

VO, vegetable oils

WG, weight gain

GENERAL INTRODUCTION

1.1. Introduction

As global landings of seafood commodities have been stagnating, if not declining, aquaculture has grown rapidly to supply the demand for seafood, circuitously, placing pressure on global fisheries by feeding wild fish to farmed fish (Deutsch et al., 2007; FAO, 2009; Tacon and Metian, 2009). Intensification of aquaculture output requires greater supply of the inputs –mainly feedstuff, their formulations and optimization. The demand for fish oil (FO) is greater in the growing aquaculture sector than for other primary industrial and human nutritional purposes (Alder et al., 2008; Miller et al., 2010; Nichols et al., 2010). The lipid component of aquafeeds, in particular for carnivorous marine species, requires inclusion of essential fatty acids (EFA) (Miller et al., 2008a; Turchini et al., 2009). EFA are the short-chain (C_{18}) and long-chain (LC, $\geq C_{20}$) n-3 and n-6 polyunsaturated fatty acids (PUFA), in particular eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6), which are required for efficient cellular metabolism and maintaining cell membrane structure and integrity (Leaver et al., 2008a; Tocher, 2010). Therefore, to sustain the aquaculture industry and reduce the pressure on natural ocean resources, FO substitution in aquafeeds for major farmed carnivorous fish species is now a reality (Tacon and Metian, 2008; Naylor et al., 2009).

This introductory chapter explains the rational for research on alternative oils to fish oil, the benefits and challenges involved in changing dietary oil, and then examines the use of potential alternative sources of n-3 LC-PUFA in aquaculture of barramundi *Lates calcarifer* Bloch (Perciformes). In the wild, this tropical euryhaline fish is endemic to the Indo-Pacific region. It is a diadromous species, returning to estuarine or marine water to breed (Greenwood, 1976). The fast-growing carnivore is produced in both freshwater and saltwater in tropical and subtropical regions of Australia, Southeast Asia and other places around the world to a harvest size of between 400 and 4000 g, depending on the production system and market. Culture of the species is well established in the Indo-Pacific region, with significant industries in Australia, Indonesia, Malaysia, Philippines, Taiwan and Thailand. Most of these industries are cage aquaculture in either natural watercourses or artificial ponds and reservoirs (Partridge et al., 2008; Carter et al., 2010; Glencross and Rutherford, 2010a).

Barramundi culture provides an interesting case of how such an adaptable fish can be grown successfully under a variety of conditions in earthen ponds, floating cages and indoor recirculation farms. Although barramundi production remains relatively small compared to many farmed species, there is great potential for global production to increase. Beginning in Thailand in the 1970s and spreading to other neighbouring countries, global production was estimated around 1700 t in the mid-1980s. By 1995, 20000 t was produced wich stabilized for several years and reached 50000 t in 2009 (Rimmer, 2003; Yue et al., 2009; FAO, 2010). Barramundi nutrition and feed formulation research started three decades ago investigating the requirements for most nutrients, energy demand, ingredient utilization and the effects of nutrition on flesh quality (Boonyaratpalin and Williams, 2002; Glencross, 2006). Optimal protein content of diets has been shown to vary with diet energy content and fish size (Catacutan and Coloso, 1995; Boonyaratpalin, 1997). Most studies have suggested a protein requirement from 45 to 55 % in the diet. For juveniles, a protein to energy ratio of 25 to 30 g/MJ is suggested. Dietary lipid requirements, for energy and EFA, show that smaller fish performed the best with a dietary lipid content of 14-16%, while growth of larger fish continued to improve with lipid content up to 19% where FO was the major dietary lipid (Boonyaratpalin and Williams, 2002; Glencross, 2006; Carter et al., 2010). As barramundi aquaculture is expanding, sustainable alternatives to FO are needed to supply this growing industry. This requires investigation to maintain the growth performance for a profitable industry and maintain nutritional and organoleptic quality at high levels for human consumers.

The aim of this chapter is to highlight the key issues relating to FO substitution in aquafeeds with emphasis on barramundi. As this thesis focuses on fish nutrition and lipid metabolism, advanced biological, chemical and genetic analyses techniques have been planned, and most of them were applied despite logistic hurdles, in order to present a comprehensive understanding of the physiological and biochemical changes occurring in barramundi fed on a range of alternative oils under various conditions.

1.2. Sustainable and responsible aquaculture

1.2.1. FISHERIES HARVEST AND IMPACT OF AQUACULTURE

The combined effects of increasing human population and increasing demand for seafood, overfishing, the expansion of aquaculture and the increasing dependency of aquaculture on FO as a major dietary lipid in aquafeeds are collectively placing pressure on wild fisheries in the oceans (Naylor et al., 2009). Fishmeal and FO are global commodities derived from the harvest of lower trophic species and have been produced for 150 years or more – long before aquaculture became a major user. The fishmeal and FO industry is centuries old, and the expansion of aquaculture in the last four decades generated an increasing demand that exceeded that made by other animal production industries (Deutsch et al., 2007; Tacon and Metian, 2008; FAO, 2009; Naylor et al., 2009). One third of the world's marine catch now goes into manufacturing fishmeal and FO to supply aquaculture, taking into account that aquaculture has been the world's fastest-growing food production industry over the last few decades (Sargent and Tacon, 1999; FAO, 2009). FO is included in aquafeeds as a source of both dietary energy and n-3 LC-PUFA. Therefore, there is currently a great need within the aquafeed industry to find and implement sustainable alternatives to FO (Tacon and Metian, 2008; Turchini et al., 2009). In recent years, substantial research has been devoted to finding, testing and developing suitable and sustainable alternatives to FO and the topic has been recently reviewed (Miller et al., 2008a; Turchini et al., 2009; Torstensen and Tocher, 2010b). The major challenge in the search for FO substitutes is to maintain fish growth and quality and the recognised dietary and health benefits from consuming fish and other seafood, due in particular to their n-3 LC-PUFA content, while simultaneously considering the importance of sustainability, economic benefits and fish welfare.

1.2.2. STRATEGIES FOR SUSTAINABLE AQUACULTURE: FEED INGREDIENT REPLACEMENT

There is currently a great and urgent need for the aquafeed industry to find and implement sustainable alternatives to FO. Worldwide, aquaculture nutrition research develops sustainable, adaptative and mitigative measures to ensure that aquaculture growth continues.

Novel and responsible strategies in sustainable feed management will assist in reducing dependence on forage fisheries to relieve the pressure placed on ocean resources. The main challenge for fish production is to maintain, if not to improve, the recognised nutritional and health benefits of fish for human consumption while simultaneously maintaining resource sustainability, fish health, product quality and the economic benefits of fish farming. In recent years, intensive research has been conducted globally to evaluate alternative lipid sources in aquafeeds (Torstensen et al., 2005; Turchini et al., 2009; Miller et al., 2010). FO demand by aquaculture is less responsive to prices than demand by the livestock industries (Delgado et al., 2003; Deutsch et al., 2007; Naylor et al., 2009). Therefore, unless appropriate and sustainable alternatives are found, growth in the aquaculture industry, and also in the competing nutraceuticals and pharmaceutical industries, is likely to push prices for FO and consequently seafood products higher. The alternatives described below have been considered highly sustainable and will be the focus of the following discussion because of their wide applications and emerging and recognised value to the aquafeed industry.

1.2.2.1. VEGETABLE OILS

In contrast to global FO production, which has levelled out in the last few decades, the production of vegetable oil (VO) has increased considerably and exceeds one hundred fold that of FO (Bimbo, 2007; Turner et al., 2008). This make oils extracted from oilseeds or other vegetable products an inexpensive and sustainable alternative to FO. However, VO does not contain the C_{20} and C_{22} n-3 LC-PUFA which are usually present in high concentrations in FO (

Table 1.1). Instead, VO are generally rich in n-6 and n-9 fatty acids, mainly linoleic acid (LA, 18:2n-6) and oleic acid (OA, 18:1n-9), with moderate or low levels of short-chain ($\leq C_{18}$) n-3 PUFA, mainly α-linolenic acid (ALA, 18:3n-3) (Guil-Guerrero et al., 2001; Aberoumand, 2009). Seed oils from some borage plants can contain relatively high content of stearidonic (SDA, 18:4n-3) at ~15% and γ-linoleic (GLA, 18:3n-6) at ~12% by weight in the Patterson's Curse (*Echium plantagineum*) shrub (Erdemoglu et al., 2004; Whelan and Rust, 2006b). Such differences in n-3 PUFA content may have numerous effects on the biology of cultured fish (Sargent et al., 2002; Miller et al., 2008a).

The quality of the edible portions of fish, especially their n-3 LC-PUFA content, was largely compromised in investigated species fed on VO instead of FO (Turchini et al., 2009; Tocher et al., 2010). Therefore, dietary n-3 LC-PUFA should be provided to cultured fish to prevent significant reductions in tissue EPA and DHA and also the n-6 LC-PUFA ARA. The provision of n-3 LC-PUFA can be achieved through different strategies: either partially replacing dietary FO with the VO, or feeding the fish on VO as a complete replacement until a certain period before reaching market size then switching to a FO based finishing diet.

Table 1.1: World production (million metric tons in 2011) and fatty acid profiles (as percentage of total fatty acids) of selected vegetable oils. (White, 2008; Rincón-Cervera and Guil-Guerrero, 2010; USDA, 2011).

Source	**Palm[1]**	**Soybean**	**Canola[2]**	**Sunflower**	**Cottonseed**	**Olive**	**Linseed[3]**	**Echium[4]**
Production	50.6	42.9	22.8	13.2	5.4	3.1	0.75	-
FA								
14:0	1.1	0.1	0.1	0.2	0.9	0.1	0.0-0.4	0.1
16:0	45.1	9.7-11.0	2.8-3.9	6.8	24.7	7.5-20.0	6.3	7.7
18:0	4.6-5.2	3.5-4.0	1.3-2.1	4.7	2.3	0.5-5.0	4-6	3.9
Total SFA	53.5	13.4	7.4	12.0	27.8	14.6-25.0	11.4	11.2
16:1n-7	0.1	0.1-0.7	0.2	0.1	0.7	0.3-3.5	0.5	0.0-0.2
18:1n-9	33.8-38.8	21.5-23.4	23.8-63.1	18.6-25.3	17.6	55.0-83.0	15-39	14.9-17.2
Total MUFA	35.1-39.0	25.0	64.7	27.2	19.2	58.5-85.0	19.6-40.0	15.5-19.9
18:3n-3	0.3	7.8	7.3-9.2	0.5-6.5	0.3	0.5-1.5	35-53	30.5-33.2
18:4n-3	nd	nd	nd	nd	nd	nd	nd	12-18
Total n-3 PUFA	0.3	8.0	12.0	6.8	0.3	0.5-1.7	56	44-47
18:2n-6	9.1	53.2	14.6-18.7	50.1-65.2	53.3	3.5-21.0	15-19	14-15.2
18:3n-6	nd	nd	nd	nd	nd	nd	nd	11.8
Total n-6 PUFA	9.4	53.5	20.1	68.0	53.5	4.0-22	15.2-19.5	26-27.5
n-3:n-6	0.0	0.2	0.6	0.1	0.0	0.1-2.5	2.7-3.8	0.9-1.6
IV[5]	45-58	125-138	97-115	122-139	99-121	76-94	170-200	198
SV[6]	195-205	188-195	168-195	186-196	189-199	184-196	188-196	191

[1] Crude palm oil; [2]low-erucic-acid rapeseed oil; [3] also known as flaxseed oil; [4] from *Echium plantagineum* seeds; [5] iodine value; [6] saponification value; nd: not detected. Trace fatty acids (<0.01) are excluded.

Feeding fish on oil rich in SDA/GLA may allow farmed fish to bypass one of the rate-limiting steps and biosynthesize LC-PUFA. The later approach, through the use of *Echium* oil (EO) or SDA-containing oil from other sources, has shown to date contrasting results due to species differences, their varied ecosystems and a changing ability for LC-PUFA biosynthesis during the different life-history stages (Tocher et al., 2006b; Miller et al., 2007a; Bharadwaj et al., 2010; Codabaccus et al., 2011). These findings indicate that further SDA feeding experiments still need to be performed on other cultured species including from different ecosystems.

1.2.2.2. BIOTECHNOLOGY PRODUCTS

Interest in producing LC-PUFA from alternative and sustainable sources for use in aquafeeds, functional foods and pharmaceuticals has fuelled recent research of LC-PUFA production in prokaryotes and single cell organisms. Practical examples of n-3 LC-PUFA production in bioreactors from thraustochytrids, diatoms and other photosynthetic microalgae achieved substantial progress in product quality as potential alternatives for FO in aquafeeds (Lewis et al., 1999; Carter et al., 2003b; Miller et al., 2010). These oils are not produced yet on a large enough scale for aquaculture and are still too expensive to be considered as practical replacements in aquafeeds (Browdy et al., 2006; Miller et al., 2008a).

There has been also increasing interest in the development of genetically modified land plants capable of producing n-3 LC-PUFA within their seeds. Heterologous reconstitution of the n-3 LC-PUFA synthesis pathway and manipulated accumulation of these products in transgenic land plants has been achieved by the reverse engineering of the primary biosynthetic enzymes. Early research was successful in producing one or two LC-PUFA from transgenic plants (Sayanova et al., 1997; Ursin, 2003). This was followed by the first report on producing SDA, EPA and DHA together in a land plant after the insertion of a variety of

genes into *Arabidopsis* (Robert et al., 2005). Major agribusiness companies are aiming to produce oils rich in n-3 LC-PUFA from transgenic crops such as soybean and safflower, but these products are still to match the n-3 LC-PUFA profile of FO. In the first quarter of 2007, one of the major agriculture biotechnology companies reported that they were not yet achieving their goal of 5% DHA in engineered oilseed crops (Damude and Kinney, 2007). Recently, a major breakthrough has occurred with the CSIRO Food Futures Flagship team achieving 5% DHA in canola seeds (Singh et al., personal communication) and considerably higher levels in a range of model plants (Petrie et al., 2010; Petrie and Singh, 2011). With the availability of high DHA-canola oil, it is estimated that one hectare of this transgenic crop will produce 1200-1500 kg of oil containing 120-150 kg of n-3 LC-PUFA. This equates to as much n-3 LC-PUFA as from 80 to 100 thousand serves (150 g in average) of farmed barramundi fed exclusively on FO.

The practical demonstration that oilseed crops can synthesise and produce unprecedented levels of n-3 LC-PUFA, especially DHA, signifies the future importance of transgenic crops as potential sustainable alternatives to FO in aquafeeds. In the oil extraction process of these novel VO, protein and genetic material is removed which will guarantee that inserted genes will not be part of the aquafeeds (Miller et al., 2010). There is a requirement for feeding and growth trials on commercial fish at each growing stage within different production conditions to evaluate the physiological effects, product quality changes and the economics of using these novel terrestrial VO in aquafeeds.

1.2.2.3. NON-FOOD MARINE ORGANISMS

Lipid and FA in marine microalgae and zooplankton contain significant amounts of PUFA. In general, red algae contain high levels of C_{20} PUFA, primarily EPA and ARA up to 27-45% and 5.3-29%, respectively (Fleurence et al., 1994; Khotimchenko et al., 2002; Li et al., 2002). These studies also found that brown algae are richer in C_{18} PUFA compared with red algae, although coming as SDA (up to 20%), which is considered a better substrate for EPA biosynthesis (Harris et al., 2007; Whelan, 2009). However, brown algae still have EPA as a

major component (8-24%) as well. Green algae have the highest C_{18} PUFA levels dominated by ALA (20-27%) then SDA and the lowest level of C_{20} PUFA. Taken together, microalgae can be considered as a promising future resource of n-3 LC-PUFA although production costs are generally too high for use in aquaculture. Current research programs looking at new sources of microalgae for biofuels may assist in bringing down production costs.

During the last two decades, research on Arctic and Antarctic zooplankton has revealed their lipid content, characteristics and FA profiles (Smith and Schnack-Schiel, 1990; Swadling et al., 2000; Nelson et al., 2001; Lee et al., 2006). The Southern Ocean krill, *Euphausia superba*, has an estimated stock biomass of 200-700 million metric tons. The high biomass reflects the ability of these predators to adapt to the seasonality in food supply (Phleger et al., 1999; 2000), with almost 6 million tons allowed catch quota for all nations (Kock, 2007; Watkins, 2007). However, less than 1% of this resource is utilized due to the remoteness of the fishery and other factors (Kawaguchi and Nicol, 2007). Our knowledge is also increasing on the lipid and FA profile of gelatinous zooplankton tunicates, the salps which can be dominant organisms in many ecosystems. Lipid from some pelagic tunicates are dominated by phospholipids with n-3 LC-PUFA, mainly EPA and DHA, representing up to 35-50% of total FA, with significant levels of SFA also present (Pond and Sargent, 1998; Jeffs et al., 2004; Mayzaud et al., 2007). Investigating other marine invertebrates such as the sponges, echinoderms and copepods has also shown interesting findings which were mainly, but not limited to, their lipid and FA composition and their content of LC-PUFA. The physio-chemical nature of these marine oils, which many contain considerable wax esters (WE), polar lipid (PL) and lipid classes other than triacylglycerol (TAG), is also under investigation to determine their suitability for processing and use in aquafeeds industry and other applications. Oils extracted from non-food marine organisms have been studied as possible alternatives to FO in aquafeeds and basic knowledge has been established on their digestibility and assimilation by some farmed fish (Olsen et al., 2004; Bogevik et al., 2010; Bogevik, 2011; Colombo-Hixson et al., 2011). The actual efficiency of utilization and changes in quality has, however, not yet been the subject of extensive studies.

Considering the potential of non-food marine organisms, the possible use of their oils can supply the growing aquaculture and pharmaceutical/neutraceutical industries. However, careful and responsible utilization plans, particularly for environmentally sensitive species

such as the Southern Ocean krill, are required so as to not impact on the interconnected trophic webs in the oceans.

Table 1.2: Average content of selected EFA (g) in 100 g raw edible portions or products of terrestrial animals and seafood at retail outlets.

Source[1]	**LA**	**ALA**	**ARA**	**EPA**	**DHA**
Terrestrial animals[2]					
Bacon (cured)	4.29	0.23	0.15	nd	nd
Beef loin steak (lean, grass + grain fed)	2.42	0.72	0.63	0.02	0.01
Chicken breast (deboned, skinless, grain fed)	0.98	0.04	0.06	0.01	0.02
Chicken egg (whole, no shell)	1.55	0.07	0.03	nd	0.06
Cow's milk (3.25% fat)	0.12	0.07	nd	nd	nd
Feta cheese (sheep milk, full cream)	0.32	0.26	nd	nd	nd
Lamb loin steak (separable lean, grass + grain fed)	2.72	1.37	0.64	0.04	0.01
Seafood[3]					
Atlantic salmon (wild, intermediate fat, Québec, Canada)	0.18	0.31	0.21	0.31	0.92
Atlantic salmon (farmed, intermediate fat, Canada, Norway and Australia)	0.93	0.16	0.15	0.96	1.84
Barramundi (wild, intermediate fat, Queensland, Australia)	0.04	0.01	0.04	0.02	0.06
Barramundi (farmed, intermediate fat, Australia)	0.07	0.02	0.05	0.07	0.15
Rainbow trout (wild intermediate fat, British Columbia, Canada)	0.23	0.15	0.11	0.17	0.39
Rainbow trout (farmed, intermediate fat, Canada and Australia)	0.67	0.12	0.05	0.28	0.64
Yellowfin tuna (canned in brine, Safcol)	0.04	0.02	0.04	0.23	0.51

[1] Content varies subject to the meat cut of the animal and analytical methods, [2] extracted data (Enser et al., 1996; Benatti et al., 2004; NUTTAB, 2010; USDA, 2010), [3] extracted data for skinless fillets (Thomas et al., 2008; NUTTAB, 2010; USDA, 2010), nd: not detected.

1.3. Fish as the main source of LC-PUFA in the human diet

Seafood is a major source of food for humans and provides a significant amount of animal protein to many communities around the world. Fish is a highly nutritious and valuable source of high quality protein and other nutrients, including n-3 LC-PUFA, for human consumption (Nichols et al., 1998; Nesheim and Yaktine, 2007). The consumption of fish has been linked to human health benefits. Indeed, oils from fish are characterized by a large range of FA ranging from 14–24 carbon atoms having 0–6 double bonds. The bulk of FA is contributed by SFA (15–25%), MUFA (35–60%) and PUFA (25–40%) (Ackman, 2007). In contrast to terrestrial dietary fats and oils, seafood lipid normally contains large amounts of EPA and DHA when alternatives to dietary FO are not substantially used (Table 1.2). Global FO production is estimated at around 1 million metric tonnes per annum (Tacon et al., 2006; Pike and Jackson, 2010). Such oils are still the least expensive natural source of preformed n-3 LC-PUFA and several industries are now specialized in their extraction and purification.

In fish tissues, the composition of FA (mainly TAG and to a lesser extent PL), is determined by diet composition and lipid metabolism (Peng et al., 2003). Fish have the ability to synthesize SFA and MUFA *de novo* and also to selectively absorb and metabolize dietary FA including n-3 LC-PUFA (Bell et al., 1997) in order to obtain an optimal fatty acid composition. This optimal composition seems to be a characteristic for each species and even strains in the case of microorganisms (Pickova et al., 1999; Petropoulos et al., 2009). Freshwater fish are generally able to elongate and desaturate ALA to EPA and DHA, whereas marine fish, which lack or have a very low activity of the Δ6 and Δ5-desaturases, cannot, and require LC-PUFA such as EPA and DHA in the diet.

1.4. PUFA metabolism in teleosts

Recent developments in nutritional biochemistry and molecular genetics provided detailed information on FA biosynthesis and the enzymes involved in LC-PUFA synthesis pathways (Gurr et al., 2002b; Chan and Vogel, 2010; Guillou et al., 2010; Liu et al., 2011). Since then, several distinct routes of LC-PUFA biosynthesis have been identified: aerobic biosynthesis in eukaryotes and most prokaryotes. Vertebrates, including fish, can desaturate 16:0 and 18:0 to palmitoleic (POA, 16:1n-7) and OA acids, respectively, but are unable to proceed past these reactions to produce ALA and LA acids as they lack Δ12 and Δ15 FA desaturases. Therefore, requirements of LC-PUFA cannot be met by *de novo* metabolic processes. Teleosts and other vertebrates must obtain LC-PUFA from their diet, ALA and LA being the short-chain dietary precursors for n-3 and n-6 LC-PUFA, respectively. Vertebrates lack the Δ15 FAD and are unable to convert LA to ALA. Moreover, ALA and LA compete for available biosynthesis enzymes required to produce LC-PUFA from each of the precursor PUFA (Gurr et al., 2002b; Wallis et al., 2002; Miyazaki and Ntambi, 2008). Each species has a different capacity to catalyse LC-PUFA from dietary precursors depending on the relative activity, availability and affinity level of enzymes involved in these reactions. Barramundi is an interesting fish for its malleable metabolism and nutritional physiology in response to alterations in ambient conditions (Katersky and Carter, 2007; Bermudes et al., 2010; Carter et al., 2010). These features open the opportunities to explore the pathways of LC-PUFA biosynthesis from different dietary precursors under optimal and less-tolerable conditions.

Two distinct desaturation enzymes; FAD6 and FAD5, were shown to be acting on the Δ6 and Δ5 positions, respectively, through the LC-PUFA synthesis pathway in teleosts except in zebrafish. These enzymes work together with several members of the FA elongase (FAE) family: Elovl5 with substrate specificity for C18 and Elovl2 for C20 and C22 PUFA (Sprecher, 2000; Hastings et al., 2001b; Hastings et al., 2005; Jakobsson et al., 2006; Monroig et al., 2009) (Figure 1.1). The first desaturation step catalysed by FAD6 on ALA and LA to produce SDA and GLA, respectively, is known as a rate-limiting step in this pathway with varying degrees occurring in the conversion of substrates to products among species (Agaba et al., 2005; Zheng et al., 2009a) (Understanding the molecular mechanisms by which nutrients and environment control transcription of desaturases and elongases has been advanced in recent years by identification of several key transcription factors that regulate lipid metabolism. For example, the transcription factors, sterol regulatory element binding proteins (SREBP-1a, SREBP-1c and SREBP-2), peroxisome proliferator activated

receptors (PPAR-α, PPAR-β and PPAR-γ), liver X receptors (LXRα and LXRβ), and carbohydrate response element binding protein (ChREBP) play key roles in the regulation of desaturases and elongases (Jump, 2008; Leaver et al., 2008a).

Table 1.3). Top carnivores have limited ability to synthesise LC-PUFA even from C18 dietary precursors and require C20 and C22 LC-PUFA directly in their diet (Rivers, 1982; Mourente and Tocher, 2009).

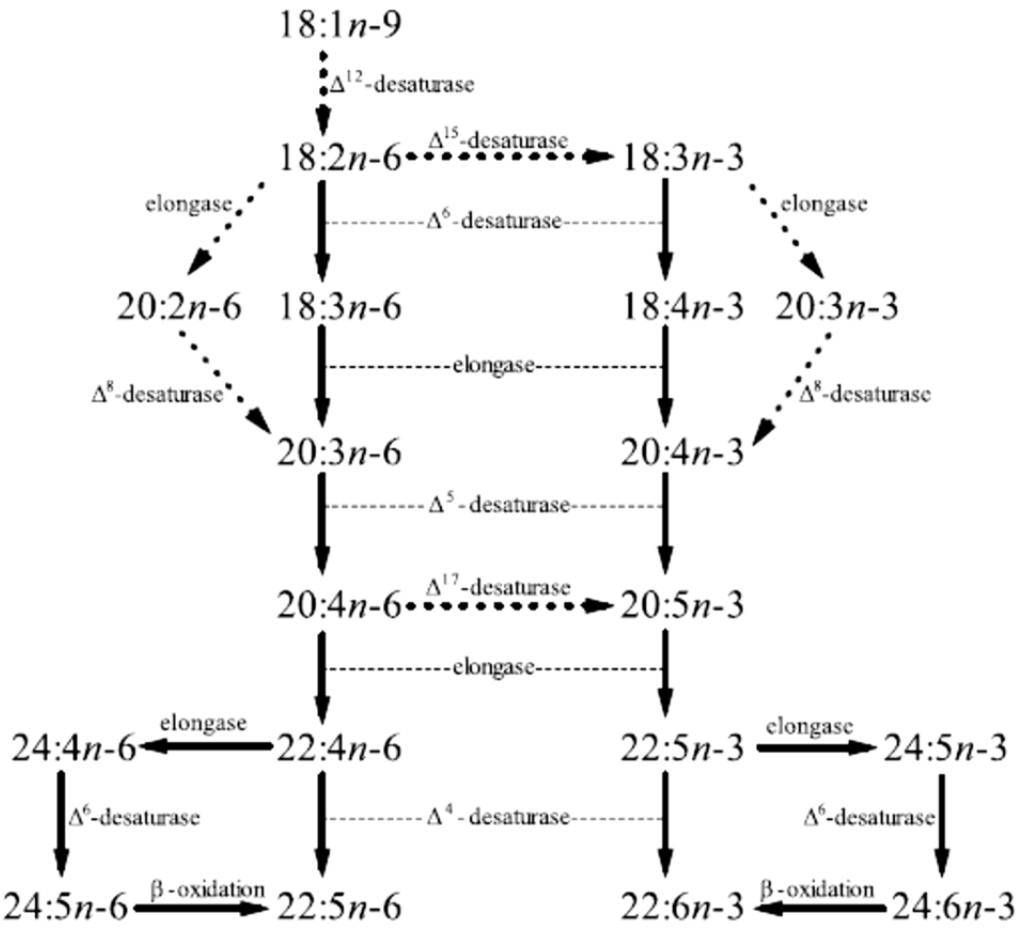

Figure 1.1: The known aerobic pathways of PUFA biosynthesis in most eukaryotes (following Sprecher, 2000; Gurr et al., 2002b; Sampath and Ntambi, 2005; Miyazaki and Ntambi, 2008; Harwood and Guschina, 2009; Li et al., 2010).

Consequently, LC-PUFA are considered EFA for vertebrates with no access to dietary ALA and LA as in geographically-isolated human populations (Gibson and Sinclair, 1981; Muskiet et al., 2004) and confined farmed fish (Glencross, 2009; Turchini et al., 2011).The final steps in the biosynthesis pathways of LC-PUFA which produce DHA from EPA and n-6 docosapentaenoic (DPAn-6, 22:5n-6) from docosatetraenoic acid (DTA, 22:4n-6) are demonstrated to occur by two distinct pathways. Sprecher (2000) suggested an elongation for EPA and 22:4n-6 followed by Δ6 desaturation, then a chain-shortening β-oxidation to reach the final product. A simpler Δ4 desaturation of EPA and DTA was demonstrated recently (Li et al., 2010) with varied conversion and substrate affinity levels.

PUFA degradation can also occur and is demonstrated principally by the liberation of C_2 (acetyl-CoA) fragments through a pathway involving oxidation at certain double bonds of specific unsaturated FA. The main forms of fatty acid oxidation are termed: α, β and ω, depending on which carbon on the acyl chain is attacked (

Figure 1.2). Of these three oxidation types, β-oxidation is the most general and prevalent. Although animal mitochondria do contain all the enzymes necessary for catabolism and are a major site for β-oxidation, other subcellular sites, such as peroxisomes are implicated. Peroxisomes contain a primitive respiratory chain where energy released in the reduction of oxygen is lost as heat. Microbodies oxidise long-chain FA to medium-chain products, which are then transported to mitochondria for complete breakdown. In this way long-chain fatty acids which are poor substrates for mitochondria, can be catabolised (Eaton et al., 1996; Wanders and Waterham, 2006).

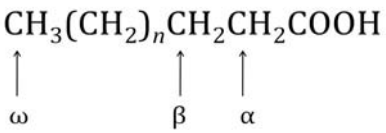

Figure 1.2: Oxidation sites and nomenclature within the fatty acid carbon chain (following Eaton et al., 1996; Wanders and Waterham, 2006).

The expression of the desaturase and elongase genes is highly regulated by many nutrients including fatty acids at the transcriptional level. The PUFA are best characterised for their ability to activate genes of fatty acid oxidation and repress genes of lipogenesis (Sampath and

Ntambi, 2005; Siddique et al., 2009). Fish physiology and ambient environmental conditions play major role in regulating LC-PUFA biosynthesis as well (Coleman et al., 2000; Ruyter et al., 2003; Jump, 2008).

Understanding the molecular mechanisms by which nutrients and environment control transcription of desaturases and elongases has been advanced in recent years by identification of several key transcription factors that regulate lipid metabolism. For example, the transcription factors, sterol regulatory element binding proteins (SREBP-1a, SREBP-1c and SREBP-2), peroxisome proliferator activated receptors (PPAR-α, PPAR-β and PPAR-γ), liver X receptors (LXRα and LXRβ), and carbohydrate response element binding protein (ChREBP) play key roles in the regulation of desaturases and elongases (Jump, 2008; Leaver et al., 2008a).

Table 1.3: Substrate conversion by Δ6 fatty acid desaturase constructs *ex vivo* from selected farmed fish.

Species	**Environment**	**Substrate**	**Product**	**Conversion %**	**Reference**
Lates calcarifer	P,F,M	ALA	SDA	32.0	(Mohd-Yusof et al., 2010b)
		LA	GLA	28.3	
Rachycentron canadum	P,M	ALA	SDA	50.8	(Zheng et al., 2009b)
		LA	GLA	36.5	
Cyprinus carpio	T, F	ALA	SDA	7.0	(Zheng et al., 2005a)
		LA	GLA	1.5	
Psetta maxima	T,C, M	ALA	SDA	59.5	(Zheng et al., 2005a)
		LA	GLA	31.2	
Sparus aurata	T, M	ALA	SDA	23.1	(Zheng et al., 2005a)
		LA	GLA	12.2	
Salmo salar	C, F,M	ALA	SDA	47.0-60.1	(Zheng et al., 2005a; Monroig et
		LA	GLA	14.4-25.0	

					al., 2010)
Oncorhynchus mykiss	C,F	ALA LA	SDA GLA	31.5 3.6	(Zheng et al., 2005a)

P, tropical; T, temperate; C, coldwater; F, freshwater; M, marine; ALA, 18:3n-3; SDA, 18:4n-3; LA, 18:2n-6; GLA, 18:3n-6.

1.4.1. EFFECTS OF DIETARY FO REPLACEMENT ON FARMED FISH

Changes in dietary FA composition resulting from replacement of FO with alternatives will directly influence lipid and FA metabolism in fish. The composition of their lipid depot and cells membrane is also affected by the uptake and transport of dietary lipid, their digestibility and endogenous capacity of biosynthesis (Turchini et al., 2009; Torstensen and Tocher, 2010a).

Compared to grow-out feeds, most starter aquafeeds fed to larvae and early juveniles have relatively low contents of lipid and have a high protein:energy ratio (Glencross and Turchini, 2010; Karalazos et al., 2010; Tocher, 2010). This is partly explained by larval and young juvenile fish having a poorer ability to digest and absorb lipid (Morais et al., 2005; Hamre et al., 2006; Cahu et al., 2009). A deficiency in dietary n-3 LC-PUFA leads to delayed metamorphosis or even a complete lack of metamorphosis in barramundi larvae as well as decreased tolerance to salinity stress (Dhert et al., 1990). EPA is also specifically required for the survival of 20-day-old larvae (Rimmer et al., 1994).

Once larvae are weaned, an adequate supply of n-3 LC-PUFA - in balance with other FA – is easily delivered to juvenile, sub-adult and broodstock fish via feeds with appropriate formulations. Early studies showed that quantitative requirements of EFA and DHA:EPA ratios vary with dietary lipid levels and fish species. This is perhaps obvious considering that these acids do not have the same physiological importance, with DHA considered as having the most importance among PUFA for cellular function of vertebrates (Watanabe, 1993;

Gorjão et al., 2009). EFA requirements have been estimated for juveniles and sub-adults of freshwater fish and indicate that these requirements can generally be met by C_{18} PUFA of both the n-3 and n-6 families at around 1% of the diet on a dry weight basis (Castell et al., 1972; Yang et al., 1994; Tocher, 2010). EFA requirements in marine and some diadromous juvenile and sub-adult fish cannot be met by C_{18} PUFA only. EFA requirements can be met by an n-3 LC-PUFA content of less than or up to 1% of the dry weight of the diet in juveniles and sub-adult red sea bream *Pagrus major* (Takeuchi et al., 1990), European sea bass *Dicentrarchus labrax* (Coutteau et al., 1996), red drum *Sciaenops ocellatu*s (Lochmann and Gatlin, 1993) and gilthead sea bream *Sparus aurata* (Kalogeropoulos et al., 1992; Ibeas et al., 1994). However, the requirement varies in relation to fish species and life-history stage. In the grow-out phase, the lipid content of aquafeeds ranged between 15 to 40% for carnivorous fish (Torstensen et al., 2004; Glencross and Turchini, 2010). Like many other fish, LC-PUFA are EFA to barramundi (Borlongan and Parazo, 1991; Boonyaratpalin, 1997). Optimal levels of LC-PUFA for juvenile and sub-adult barramundi were reported at 1.5-2% in a diet that contained 13% lipid (Wanakowat et al., 1993; Williams et al., 2003; Glencross and Rutherford, 2011) with a recommended n-3:n-6 PUFA ratio of 1.5–1.8:1 for higher growth rates (Williams et al., 2006).

As discussed above, most VO are dominated by LA and ALA, with both FA passing through the rate-limiting FAD6 step before being subjected to further elongation and desaturation to reach the biologically more important LC-PUFA – EPA and DHA. Lipid metabolism studies of Atlantic salmon liver indicated that LC-PUFA biosynthesis genes were up-regulated in VO-fed fish compared to the FO treatment (Jordal et al., 2005; Leaver et al., 2008b). In euryhaline and diadromous fish, diet has been shown to influence LC-PUFA metabolism on a gene level more than salinity (Zheng et al., 2005c; Li et al., 2008). The previous studies have indicated increased gene activity of the n-3 LC-PUFA biosynthesis pathway when these FA are lacking in the fish diet, however, increased accumulation of the n-3 LC-PUFA has generally not occurred. Different species of fish from different ecosystems appear to strive, at least at the gene expression level, to biosynthesize LC-PUFA from available dietary precursors when FO is replaced, but the limited biosynthesis ability precludes higher levels of production that result in increased accumulation. In some fish, replacement levels of up to 75-100% with alternative lipid in the grow-out phase showed positive outcomes, while selected studies showed that FO cannot be replaced totally for other species. Growth performance of farmed fish was either reduced (Glencross et al., 2003a; Izquierdo et al., 2003; Peng et al.,

2008) or not significantly affected when VO and animal fat were fed as alternatives to FO to tropical, temperate and coldwater species (Bransden et al., 2003; Bahurmiz and Ng, 2007; Miller et al., 2007a; b; Shapawi et al., 2008). These variations are mainly attributed to the quality of the FO alternatives which may contain anti-nutritional factors that can depress fish growth. The fatty acid composition of fish is predominantly a reflection of the FA composition of their diet (Glencross et al., 2003b; Mourente and Bell, 2006; Miller et al., 2008a). The capacity of the n-3 LC-PUFA pathway to produce FA that are at low levels only or are not available in the diet is limited in altering overall FA composition in fish. Replacing FO with VO in aquafeeds will directly decrease dietary EPA, DHA and ARA, while increasing OA, LA and ALA. These differences will have several effects on the sensory and organoleptic quality of fish as well as their metabolism, and ultimately their health benefits to human consumers. Therefore, there is a need to understand the changes in LC-PUFA composition and biosynthesis in barramundi, as a major tropical farmed fish, when fed VO as a substitute for FO and across a range of environmental conditions.

1.4.2. LIPID CONTENT AND QUALITY OF CULTURED FISH

Changes in dietary FA may directly influence fish lipid metabolism and therefore tissue lipid composition with other environmental factors also influencing lipid metabolism - FA biosynthesis and β-oxidation, lipid transport, uptake and digestibility (Turchini et al., 2009; Torstensen and Tocher, 2010b). Changes in the overall metabolism due to FO replacement with VO at the level of total lipid and protein in the whole fish have been shown (Panserat and Kaushik, 2010; Torstensen and Tocher, 2010b). However, such replacement did not significantly affect the content of total lipid and protein in fish muscle (Bendiksen et al., 2003; Torstensen et al., 2005; Turchini et al., 2009). Such replacement can manipulate the lipid class ratios, mainly TAG:PL, in Atlantic salmon *in vivo* and *in vitro* (Nanton et al., 2007; Todorčević et al., 2008). Lipid storage was not always shown to be affected in different tissues, as in the case of adipose, but fatty livers have been reported from fish fed on VO as a partial or complete replacement for FO (Torstensen et al., 2000; Jordal et al., 2007). Such changes in liver lipid content have been attributed to the imbalanced dietary FA, low dietary

n-3 LC-PUFA combined with other environmental effects disrupting physiological function in this major lipid metabolism organ.

Following partial or complete FO replacement with VO, fish tissues respond differently to the dietary change. Some fish selectively retain and store n-3 LC-PUFA in their tissues if their diet was low in these compounds (Izquierdo et al., 2003; Stubhaug et al., 2007). This preferential order in utilizing dietary components indicates the biological importance of n-3 LC-PUFA for fish and the priorities for using them in structural and physiological roles rather than energy production (Bell et al., 2003; Stubhaug et al., 2007). To this end, the most evident effect of FO replacement with alternative oils is the direct effect on FA composition of the fish flesh and its resulting nutritional quality, particularly the content of n-3 LC-PUFA. Nevertheless, many researchers have showed that farmed fish can still be considered a valuable source of EPA and DHA even when a high FO replacement regime is applied (Rosenlund et al., 2010).

Despite the numerous studies so far, it is not yet confirmed how the changes in dietary lipid can affect the fish flesh quality as measured by sensory and organoleptic characteristics, colour, texture and gaping, shelf life and flavour. Although less effect was reported on fillet texture and storage stability (Mørkøre and Einen, 2003; Ng and Bahurmiz, 2009), flesh colour (Regost et al., 2004; Izquierdo et al., 2005) and aroma (Sérot et al., 2001; Turchini et al., 2004) were influenced by the dietary lipid source and sometimes by the varied sources of VO. A range of studies have shown that up to 50-100% of dietary FO can be replaced with VO without compromising the fillet texture of farmed fish (Rosenlund et al., 2001; Menoyo et al., 2004; Ng and Bahurmiz, 2009) which contradicted other reports (Andersen et al., 1997; Regost et al., 2004). These contrasting observations indicate that our current understanding in this area is limited and further research is needed keeping in mind the range of individual preferences and subjective choices between consumers that judge the quality of farmed fish fed FO alternatives.

1.4.3. EICOSANOID PRODUCTION

One of the effects of dietary PUFA on immune modulation is occurring through the production of eicosanoids derived from phospholipid in cellular membranes. Eicosanoids are metabolites of the C_{20} PUFA, namely ARA and EPA, and are formed through a set of

enzymes including cyclooxygenase and lipoxygenase. They have a central role in immunity functions and inflammatory responses as well as regulating the expression of several genes (Calder, 2006; Galli and Calder, 2009). Eicosatetraenoic acid (ETA, 20:4n-3) and dihomo-γ-linolenic acid (DGLA, 20:3n-6) are also considered precursors for eicosanoids in fish (Bell et al., 1994; Ghioni et al., 2002a). The availability of these LC-PUFA is determined by their presence in the diet or the endogenous ability of the fish to synthesize them efficiently from their precursors.

The use of dietary VO has implications on fatty acid composition of fish and therefore eicosanoid metabolism and production and their resulting immunity and stress response. When FO was replaced by VO in aquafeeds, nonspecific immune parameters and eicosanoid production in fish were significantly affected in comparison with a FO diet (Bell et al., 1996; Bransden et al., 2004). It also appears that the effect of dietary VO on prostaglandin production depends on the type of VO and its FA composition. For instance, the inclusion of oils rich in n-3 PUFA generated higher level of the prostaglandins of the 3-series and leukotrienes of the 5-series, whereas dietary VO rich in n-6 PUFA increase the production of prostaglandins of the 2-series and leukotrienes of the 4-series (Ashton et al., 1994; Balfry and Higgs, 2001). The level of FO substitution with VO has been also reported to have an inverse effect on eicosanoid production in farmed fish. A diet with capelin oil substituted (in half) by soybean, rich in LA, increased prostaglandin E_2 in Atlantic salmon (Gjøen et al., 2004), while linseed oil, a major source of ALA, reduced the production of prostaglandin E_2 and thromboxane B_2 which increased the anti-inflammatory activity (Bell et al., 1993). Moderately increased dietary ALA in Atlantic salmon fed a diet containing a blend of VO, made up of rapeseed oil, palm oil and camelina oil, resulted in a slight reduction in plasma prostaglandin E_2 levels (Petropoulos et al., 2009).

Alterations in the ARA:EPA ratio can result in imbalances in eicosanoid production and affect the immunity and stress response if fish. When dietary LA is desaturated and elongated to DGLA, eicosanoid production is impaired in marine fish cells as EPA-derived prostaglandins and leukotrienes can suppress the production of similar products derived from ARA (Bell et al., 1994; Bell et al., 1996). When Atlantic salmon was fed exclusively on VO, the EPA:ARA ratio decreased three-fold in leukocyte membranes and the production level of several eicosanoids was lowered compared to fish fed on FO (Bell et al., 1996). However, a well-formulated VO-based diet can keep the dietary FA ratio in balance with less negative effects on fish immunity and less altering of the EPA:ARA ratio in their leukocyte

membranes. Some VO, and oils from sources other than FO, have unique FA profile and n-3:n-6 ratios which make them of considerable potential for feeding farmed fish due to their ability to maintain the general health condition and eicosanoid production levels.

1.5. Environmental regulation of lipid metabolism in fish

Fish species have evolved within different ecosystems where they have developed varied nutritional physiologies and metabolic capabilities under specific biotic and abiotic conditions. The effects of trophic level and the inclusion of alternatives to FO in the diet on growth performance have been discussed earlier in this review. Abiotic factors, such as water salinity or temperature, also have major impacts on regulating lipid metabolism in fish (Hazel, 1995a; Miller et al., 2008a). A meta-analysis review quantified a range of independent factors on the growth of fish fed alternatives to FO. Amongst compared factors, water salinity and oil source were related to fish size and shown to significantly influence on fish performance (Sales and Glencross, 2011). Aquaculture experiments are usually conducted under diverse designs to examine response to changes in one factor or more while other conditions are controlled (Astles et al., 2006; Sae-Lim et al., 2010). In nature or under farm conditions, the interaction between diet and environment is more complex. However, factorial experiments provide the foundation for understanding modulation of lipid metabolism under changing conditions and the level of interaction between certain factors set to resemble the farm situation (Torstensen et al., 2001; Le Boucher et al., 2011; Trabelsi et al., 2011). Chapter 2, 3, 4 and 5 in this thesis are investigating the interaction between the diet, in terms of its lipid source, and different environmental factors such as salinity, temperature or pathogen infection.

Change in salinity or temperature cause changes in feed intake, growth efficiency and lipid metabolism in fish such as European sea bass (Cordier et al., 2002; Eroldoğan et al., 2004; Grigorakis, 2007) and gilthead sea bream (Ibarz et al., 2005; Grigorakis, 2007). In comparison, eurythermal or euryhaline species like barramundi exhibited efficient growth performance across a range of environments (Harpaz et al., 2005; Katersky and Carter, 2007). It would be particularly useful for the lipid metabolism of these species to be investigated within their tolerable range of conditions. Understanding the levels of interactions between

the environment and biological factors on fish growth and quality – termed biochemical composition (Figure 1.3), and its influence on regulating the dynamics of lipid metabolism, will assist in maintaining fish product quality and improving aquaculture management practices.

Barramundi inhabit a wide variety of habitats in rivers, creeks, mangrove estuaries and coastal areas in clear to turbid water throughout latitudes spanning between Asia and sub-tropical Australia (Rimmer, 2003; Bermudes et al., 2010). They adapt to varying environmental conditions during different seasons and stages of their life-cycle (Balston, 2009; Carter et al., 2010; Newton et al., 2010), and tolerate extreme experimental conditions

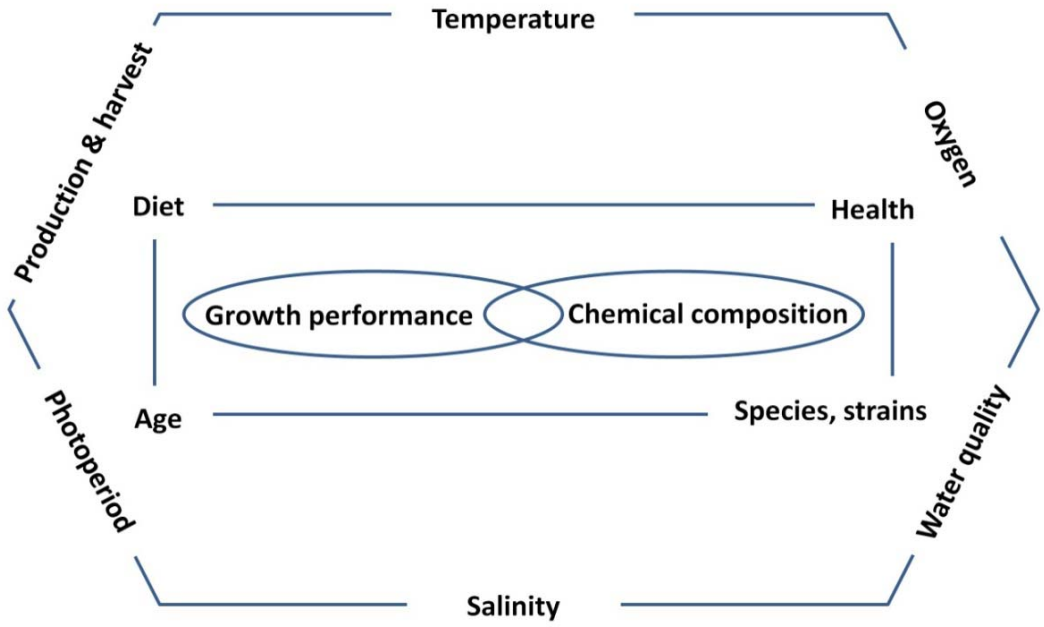

Figure 1.3: Farmed fish growth performance and chemical composition as affected by the principal known biotic (inner rectangle) and the major studied abiotic factors (outer hexagon) (modified after Cossins and Crawford, 2005b; Hargrave, 2005; Deane and Woo, 2009; Grigorakis, 2010).

of high or sub-optimal temperatures (Katersky and Carter, 2005; Williams et al., 2006) and hypersalinity (Salama, 2008) showing malleable metabolism and nutritional physiology. Therefore, investigating lipid metabolism of barramundi provides an exceptional opportunity to understand the interaction between the environment and diet and establish another perspective for comparison with temperate or cold-water and stenohaline farmed fish.

1.5.1. ABIOTIC FACTORS

1.5.1.1. TEMPERATURE

Temperature has a primary influence on physical properties of membrane and storage lipids and their composition in ectothermic animals such as fish (Buda et al., 1994; Käkelä et al.,

2008). The capacity for lipid storage increases in cold-adapted and cold-acclimated fish (Pörtner, 2002; Guderley, 2004a). Fish modify the composition and unsaturation level of their membrane lipids in response to changes in environmental temperatures, within their thermal tolerance range (Hölttä-Vuori et al., 2010; Ibarz et al., 2010). This plasticity is known as homeoviscous adaptation, a complex molecular and biochemical phenomenon by which ectotherms maintain fluidity in their cell membranes independent of the surrounding temperature (Hazel, 1995a; Hofmann and Todgham, 2010). Cold-challenged ectothermic teleosts displayed significant up-regulation in their FAD (Tiku et al., 1996; Ruyter et al., 2003; Gracey et al., 2004) and FAE gene expression *in vivo* and *in vitro* (Tocher and Sargent, 1992; Ruyter et al., 2003).

The capacity of fish to adapt to any sustained or acute environmental stress, such as change in temperature, can be affected by nutritional history. A 3 month deficiency in dietary n-3 LC-PUFA did not drastically impair the capacity of European sea bass juveniles to adapt to high temperature (Person-Le Ruyet et al., 2004). Changes in water temperature lead to alteration in the fatty acid composition of the TAG depots and PL structuring the cell membranes with increases in the levels of FA unsaturation with decreasing temperature. However, this process occurs differently between species and across tissues and depends on many factors such as season, temperature, fishing ground, fish species, age, gender or nutritional habits (Gamez-Meza et al., 1999; Tanakol et al., 1999). The major adaptation to decreasing temperature occurs in the PL fatty acids (PLFA) within the cell membranes, while FA within TAG are also affected, but to a lesser extent than PLFA. A major effect of temperature change is on lipid digestibility in stenothermal fish. Coldwater fish were less efficient in digesting dietary SFA, then MUFA then PUFA when they were raised at a water temperature above their optimal levels (Olsen, 1998; Ng et al., 2004; Ng et al., 2010) which will imbalance the energy intake. In fish, increasing FA digestibility occurred with increased degree of unsaturation, and decreased digestibility occurred with increasing chain length (Olsen and Ringø, 1997a; Johnsen et al., 2000; Ng et al., 2003). Lowering water temperature may cause some dietary oils to solidify in the digestive tract of fish (Olsen and Ringø, 1998). Thus, the availability and absorption of dietary lipid is strongly influenced by water temperature for fish. Chapter 4 in this thesis will investigate the changes in lipid and FA composition in barramundi fed on oils from different sources and with the fish subjected to sub-optimal temperature. This will develop the current understanding on the ability and limitations of tropical fish to cope with

changes in temperature, and the affects of these changes on the growth, physiology and quality of farmed tropical fish.

1.5.1.2. SALINITY

Different species of fish may spend different stages of their life-history in freshwater, marine and estuarine ecosystems. In order to reproduce, anadromous species migrate from sea to fresh water, while catadromous fish spend most of their life in fresh water and migrate to sea. Diadromy may have evolved in fish as a means to take advantage of the favourable feeding conditions in the ocean (Gross et al., 1988; McDowall, 2008). Physiological changes associated with the diadromous habit have major influences on lipid metabolism, FA requirements and the resultant FA profile of the fish (Tocher, 2010). The rearing salinity of farmed fish affects their general physiology, osmotic regulation, lipid digestibility and, consequently, the biochemical and FA composition (Partridge and Jenkins, 2002; Haliloğlu et al., 2004; Jana et al., 2006). Lipase mediates lipid depletion in acclimation to salinity change, while thyroxine, cortisol, growth hormone and prolactin underlie alterations in lipid metabolism associated with salinity change (Sheridan, 1989; Jarvis and Ballantyne, 2003).

Changing salinity had a significant effect on FA profile, mainly PUFA and the n-3:n-6 ratio, in fish. Early reports showed dramatic decreasing of the n-3:n-6 ratio of sweet smelt, *Plecoglossus altivelis*, when they migrated from freshwater to seawater (Ota and Takagi, 1977), while the ratio increased in masu salmon, *Oncorhynchus masu*, migrating from rivers to the sea (Ota, 1976). Barramundi can be ascribed to specific environments by examining their fatty acid profile where n-3:n-6 is higher in samples collected from saltwater compared with freshwater habitats, which is related to their natural diet (Bandaranayak et al., 2004). Other research found that diadromous or euryhaline fish have a higher n-3 LC-PUFA content and their ability to synthesize these FA from dietary precursors changes through the life cycle as in Atlantic salmon (Miller et al., 2008a). Salinity changes manipulated the n-3 LC-PUFA content in milkfish, *Chanos chanos* (Bautista et al., 1991; Borlongan and Benitez, 1992), rabbitfish (Li et al., 2008) and Japanese sea bass (Xu et al., 2010).

There are other molecular, evolutionary and dietary factors contributing to the different capacities of marine, freshwater or diadromous fish to endogenously biosynthesize n-3 LC-

PUFA as discussed earlier (section 1.4). Together with changing salinity across or through the fish life cycle, or the culture period in farms, these factors can alter FA composition and n-3 LC-PUFA levels in complicated and networked interactions that still need to be understood better. Chapter 2 and 3 investigated the effect of salinity on lipid and FA metabolism in the whole body and tissues of barramundi fed on alternatives to FO. In its wider scope, this contribution will advance our knowledge on FA metabolism in euryhaline fish as a result of the interaction between diet and environment.

1.5.2. BIOTIC FACTORS

Narrative and analytical reviews on the effects of FO replacement with VO described strong responses of growth and FA composition which were controlled by species differences, size, tissue integrity and function, health and disease resistance and the complex relationships occurring between all these factors (Waagbø, 2006; Montero and Izquierdo, 2010; Sales and Glencross, 2011). Other factors also play key roles in regulating lipid metabolism, such as sex (Batista-Pinto et al., 2009; Ma et al., 2011), stage in life cycle (Tsai et al., 2008; Tocher, 2010) or behaviour (Anttila et al., 2010; Ozorio et al., 2010). Of the many intrinsic biotic factors affecting fish lipid metabolism, this thesis will investigate and discuss the interaction of diet with the immunity status and size of barramundi.

1.5.2.1. DISEASE

The role of lipid, FA, and their metabolites in the health of cultured fish has been intensively studied both *in vivo*, following feeding them different oil sources and ratios of lipids, and *in vitro* within different incubation conditions (Tocher, 2003a; Montero and Izquierdo, 2010). However, our understanding on how lipid and FA profiles will change in pathogenically challenged fish is still limited. Modulation of the immune system induced by changes in dietary fatty acids depends on several biological and methodological factors, such as the type and concentration of fatty acids, cell types, species of experimental animal, serum used in the *in vivo* or *in vitro* cultures, among others (Calder, 1999). Mechanisms involved in the

stimulation of the fish immune system by FA and the regulation of immune-response genes by dietary FA are poorly understood. Indeed, manipulation of the dietary n-3:n-6 ratio has been shown to affect fish resistance to pathogens (Kiron et al., 1995; Balfry and Higgs, 2001; Bransden et al., 2003). Replacing FO by terrestrial VO in aquafeed has been observed to affect immune parameters of farmed fish such as the phagocytic activity of head kidney macrophages and FA composition of immune cells (Farndale et al., 1999; Montero et al., 2003) or eicosanoid production (Ganga et al., 2005; Mourente et al., 2005). However, little is known about the effect of dietary FA on other processes, such as those related with viral, parasitic and bacterial infections.

Parasitized fish showed profound lipid profile modifications with reduced total lipid content in tissues and changed lipid class composition (Broek, 1978; Blanar et al., 2005; Schaufler et al., 2008). Cholesterol and TAG were reduced, while PL and non-esterified fatty acids increased in parasite-infected fish. It has been suggested that the parasite may be utilizing some lipids from the host, while the host fish are depleting and synthesizing other lipid classes in response to infection (Schaufler et al., 2008). Bacterial infections also have been shown to seriously affect lipid content in aquatic invertebrates. Shrimp, *Penaeus vannamei*, lobster, *Homarus americanus*, and sea scallops, *Placopecten magellanicus*, infected with pathogenic bacteria had their lipid depots (TAG levels) reduced compared with uninfected animals (Stuck et al., 1996; Floreto et al., 2000; Pernet et al., 2006). Basic and comprehensive research on the dynamics of changes in fatty acid and lipid class profiles as a result of bacterial or viral infection in finfish are still scarce. Chapter 5 is presenting a link between barramundi resistance to pathogens and dietary lipid with a focus on FO alternatives. Results reported in Chapter 5 will expand our understanding on the interactions between lipid content and composition and fish health.

1.5.2.2. WEIGHT

Fish weight at different life-history stages is known to impact on FA composition of tissues and is driven by changes in growth rates, nutrient requirement, feeding habits, the contribution of breeding and other factors (Muyonga et al., 2008; Hosseini et al., 2009; Mayzaud et al., 2011). Growth performance and composition parameters of different fish species fed VO replacing FO showed significant correlation between fish weight and measured responses. Replacing FO completely by VO in aquafeeds proved quantitatively to

have a negative impact on growth of most fish species (Sadler and Imrie, 2010; Sales and Glencross, 2011). There is a need to establish specific understanding on changes in lipid content and FA composition in different sizes of barramundi fed different dietary oils compared to FO. In Chapter 7, barramundi growth has been correlated with their weight and dietary content and composition of LC-PUFA and an initial meta-analysis perspective is presented for their growth rates as influenced by diet.

1.6. Stimulating lipid metabolism by bioactive ingredients

Several bioactive compounds, such as sesamin and lipoic acid, are lipid modulators and have been well-investigated in mammalian systems (Ashakumary et al., 1999; Mizukuchi et al., 2003; Ide et al., 2004; Huong and Ide, 2008). Sesamin inclusion in an ALA-rich diet increased DHA in TAG and PL of rainbow trout (Trattner et al., 2008b) and in PL of Atlantic salmon parr (Trattner et al., 2010). In Atlantic salmon hepatocytes, it was shown that sesamin increased the synthesis of radiolabelled ALA towards DHA (Trattner et al., 2008a). The metabolic effects of sesamin have been suggested to be caused through the activation of PPARs and SREBP-1 (Ashakumary et al., 1999; Ide et al., 2004). Furthermore, sesamin has been reported to inhibit cholesterol absorption and synthesis, and tocopherol hydroxylation and clearance in mammals (Sirato-Yasumoto et al., 2001; Jeng and Hou, 2005). However, less in known on the dynamics of lipid modulation by sesamin in farmed fish, which are becoming major sources of n-3 LC-PUFA for human nutrition. Lipoic acid also was reported to have several effects on lipid metabolism in poultry (Hamano, 2006) and rodents (Mythili et al., 2006), while its use increased polar lipid EPA in the muscle and brain of South American pacu, *Piaractus mesopotamicus* (Trattner et al., 2007).

The opportunity exists and is clearly needed in the farming of fish to enhance the activity and conversion of key intermediates along the LC-PUFA pathway. A number of approaches for the inclusion of LC-PUFA precursors and / or bioactive compounds in aquafeed can be considered with further investigation required *in vitro* and *in vivo* on farmed fish. Chapter 6 investigated the effect of sesamin in combination with VO on modulating the lipid profile and LC-PUFA levels in juvenile barramundi as a potential strategy to enhance the quality of farmed fish fed on alternatives to FO.

1.7. Aims and organisation of thesis

The research undertaken in this thesis examined feeding barramundi, a tropical euryhaline fish widely cultured in tropical and subtropical Asia and Australasia, on novel oils as replacements for FO. The overall aims included to investigate the effects of environmental variables when barramundi were fed novel FO alternatives. Response criteria were growth, lipid content and composition, with emphasis on the health-benefitting n-3 LC-PUFA, and fatty acid metabolism. The implications of these changes on fish flesh composition and the importance of enhancing levels of n-3 LC-PUFA available for human consumption motivated this research.

As most barramundi farms are based on outdoor ponds and are exposed to fluctuations in environment, the experiments conducted tested dietary alternatives in relation to a wide range of salinity, temperature and unfavourable health conditions. These experiments investigated the following:

Feeding juvenile barramundi VO rich in SDA and GLA, as precursors for n-3 and n-6 LC-PUFA, was performed in 4 feeding experiments. Fish were raised in either fresh water or sea water and the subsequent effect on their growth and FA biosynthesis at both a whole body and tissue level was determined. An EO formulated diet replacing FO was examined in comparison to a diet made with RO as a negative control. These trials aimed to investigate the biochemical capacity of barramundi to synthesise and accumulate TAG and PL n-3 and n-6 LC-PUFA under different conditions of salinity (Chapters 2 and 3).

A diet rich in SDA and GLA from EO can probably contribute more efficiently, compared to RO lacking these fatty acid, for the lipid changes required when barramundi, a representative to tropical ectotherm, is under thermal stress. Therefore, the effects of sub-optimal temperature on changes in lipid and fatty acid metabolism in the whole body and tissues of barramundi following feeding on different dietary precursors for LC-PUFA were studied (Chapter 4).

As biotic factors can also affect LC-PUFA biosynthesis from dietary precursors, the modulation of LC-PUFA metabolism and accumulation in fish fed EO as a result of bacterial

infection was investigated. The interaction between dietary lipid rich in LC-PUFA precursors on barramundi immunity, fatty acid metabolism and LC-PUFA metabolites following pathogenic bacterial infection will provide better understanding for the use of FO alternatives in aquaculture (Chapter 5).

Several plant-derived bioactive compounds, such as sesamin, can stimulate certain lipid metabolism pathways. Therefore, the effect of combining sesamin with dietary ALA from LO, SDA and GLA from EO or preformed n-3 LC-PUFA in FO are compared. This is aimed to assist in understanding the modulation of fatty acid composition and LC-PUFA biosynthesis by addition of sesamin in diets of barramundi (Chapter 6).

The chapters of this thesis have been published or are under review for peer-reviewed journals in the fields of aquaculture, nutrition, lipid chemistry and biochemistry as listed at page iii and this will also be noted for each chapter within the thesis.

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CHAPTER 2 -

Alhazzaa, R., Bridle, A.R., Nichols, P.D. and Carter, C.G. (2011): Replacing dietary fishoil with Echium oil enriched barramundi with C18 PUFA rather than long-chain PUFA. *Aquaculture* 312, 162-171.

CHAPTER 3-

Alhazzaa, R., Bridle, A.R., Nichols P.D. and Carter, C.G. (2011): Up-regulated desaturase and elongase gene expression promoted accumulation of polyunsaturated fatty acid (PUFA) but not long-chain PUFA in Lates calcarifer, a tropical euryhaline fish fed a stearidonic- and γ-linoleic acid enriched diet. *Journal of Agricultural and Food Chemistry* 59, 8423–8434.

CHAPTER 4

Alhazzaa, R., Bridle, A.R., Nichols, P.D. and Carter, C.G.: Tropical ectotherm coping with sub-optimal temperature: modifications in fatty acid influenced by dietary lipid. *In preparation*.

CHAPTER 5

Alhazzaa, R., Bridle, A.R., Mori, T, A. and Barden A, E., Nichols, P.D. and Carter, C.G.: Dietary lipid modulation of fatty acid composition and immunity in barramundi, *Lates calcarifer*, following disease challenge. *In preparation.*

CHAPTER 6-

Alhazzaa, R., Bridle, A.R., Nichols, P.D. and Carter, C.G. (2012): Sesamin modulation of lipid class and fatty acid profile in early juvenile teleost, *Lates calcarifer*, fed different dietary oils. *Food Chemistry* 134, 2057–2065.

8. APPENDIX

This appendix contains supplementary information from Chapters 4 and 5.

These chapters and appendix 8 have been removed for copyright or proprietary reasons

GENERAL DISCUSSION

7.1. Overview

Recent extensive reviews on fish oil (FO) replacement in aquafeeds reflect a scarcity in research on feeding FO alternatives to marine carnivorous fish from tropical regions compared to species from cold or temperate environments (Turchini et al., 2009; Sales and Glencross, 2011). Farming of tropical fish encompasses a range of species and contributes significantly to global aquaculture production (De Silva, 1998; Troell, 2009). This thesis investigated the use of selected vegetable oils as alternatives to FO in barramundi, *Lates calcarifer*, aquafeeds as an important tropical euryhaline farmed fish. Barramundi is a fast-growing, euryhaline and eurythermal farmed fish of considerable commercial interest for its nutritious value for human consumption (Katersky and Carter, 2007; Bermudes et al., 2010; Carter et al., 2010).

A series of experiments were designed to understand the changes in lipid metabolism in barramundi, focusing on n-3 long chain ($\geq C_{20}$) polyunsaturated fatty acid (n-3 LC-PUFA) composition, under varied salinity, temperature and immunity stress. A further experiment examined the stimulation of lipid metabolism, also with emphasis on n-3 LC-PUFA production, following addition of a plant-derived bioactive compound. Hypotheses were tested through a combination of experimental designs applying analytical, biochemical and molecular techniques. The main focus was to investigate the endogenous capacity of barramundi to synthesize n-3 LC-PUFA from their short-chain and less unsaturated C_{18} precursors and to understand the underlying physiology and biochemistry. Chapters 2 to 6 examined the main challenges associated with changing the dietary oil source of aquafeeds to vegetable oils that are more renewable and of lower production cost in the long run compared to FO (Lam et al., 2009; Turchini and Mailer, 2010). Selected VO, varying in fatty acid (FA) composition, were fed to barramundi under different biotic and abiotic conditions to assist in understanding the interactive effects between dietary lipid and ambient conditions on fish physiology and PUFA metabolism. Barramundi were also fed on VO in combination with sesamin to modulate lipid metabolism and increase n-3 LC-PUFA biosynthesis.

The following general discussion reviews the results, the opportunities and the limitations obtained from these experiments in order to determine the potential for use of alternative n-3 LC-PUFA sources for barramundi aquaculture. The overarching aim was to maximise the

endogenous synthesis of n-3 LC-PUFA from their dietary precursors, while maintaining fish growth and product quality as compared with feeding on FO. Previous research showed varied levels of quality and growth alterations when FO was replaced in aquafeeds for carnivorous fish (Chapter 1). This chapter will contribute further to this discussion by comparing the current findings for barramundi with those for migrating or non-migrating species from cold- or temperate-water ecosystems.

A limited number of fish species have been used in the research fields of lipid metabolism, gene-diet and diet-environment interactions (Leaver et al., 2008; Hölttä-Vuori et al., 2010). Experiments presented in this thesis covered different aspects of diet-environment interaction and its effect on barramundi lipid metabolism. Although barramundi is not commonly used in molecular and biochemical research, malleable responses in lipid metabolism to alterations in ambient conditions make this teleost a valuable model to explore nutritional physiology and metabolism.

7.2. Alternatives to FO in barramundi aquaculture

Optimal levels of total lipid in barramundi aquafeed are relatively high, with FO used as the major source of oil until recently (Chapter 1). As with many other fish, vegetable oils (VO) have potential to be practical alternatives to FO in barramundi aquafeed. VO, with high MUFA content, can probably satisfy the energy demand of barramundi, but the lack of LC-PUFA in VO may impair fish physiology and growth. Serial replacement of dietary FO with soybean oil (SO), rapeseed oil (RO) or linseed oil (LO) showed that growth performance was significantly reduced by complete replacement of FO with VO (Raso and Anderson, 2003). In the same experiment, feeding a range of FO and VO blends (0.75:0.25, 1:1, 0.25:0.75 ratios) to 19 g barramundi resulted in comparable growth efficiency. This comparison suggests that, while barramundi juveniles receive fishmeal and casein (6:4 ratio) as protein source, VO cannot completely replace FO without compromising their growth. In contrast, the current thesis demonstrated that complete replacement for FO with VO did not affect the growth of barramundi at different sizes as long as only fishmeal - in this case which was defatted - is the only source of protein. In a shorter (15 days) experiment starting with smaller (4 g) fish, replacing fishmeal with 45% lupin meal (mixture of kernel and protein concentrate) did not

compromise barramundi growth (Katersky and Carter, 2009). The growth of bigger (70 g) fish was enhanced by replacing fishmeal completely with lupin protein concentrate, lupin kernel meal, wheat gluten, rapeseed (canola) meal or poultry offal meal (Glencross et al., 2011). Both of the abovementioned experiments used FO as a lipid source and the diets satisfied recommended energy and protein requirements for barramundi. The earlier results, taken together with the ones recorded in this thesis, raise questions about nutrient utilisation and possible synergistic effects of combined use of non-marine alternatives for fishmeal and FO in aquafeed formulations that may negatively affect barramundi growth performance. Combined high levels of replacement of fishmeal and FO with terrestrial plant-origin alternatives reduced the growth of cobia, *Rachycentron canadum* – a tropical carnivorous fish (Salze et al., 2010), and in Atlantic salmon, *Salmo salar* (Torstensen et al., 2008) and rainbow trout, *Oncorhynchus mykiss* (Panserat et al., 2009); the latter two species are both coldwater carnivorous fish.

A strategy to completely replace dietary FO with VO was adopted in this project under standardized rearing conditions. VO tested in barramundi diets had comparable effects to FO on growth efficiency, apparent digestibility of the oils and the total lipid content of fish whole body and tissues (Chapter 2 and Chapter 3). However, VO reduced the content of both n-3 and n-6 LC-PUFA and the n-3:n-6 ratio regardless of abiotic (Chapter 2,Chapter 3 andChapter 4) or biotic (Chapter 5) conditions. Moreover, other measured parameters, such as the concentration of eicosanoid metabolites in blood plasma, were significantly compromised following feeding on VO (Chapter 5). Stimulating lipid metabolism of fish fed VO with sesamin lead to a significant increase in relative levels of n-3 LC-PUFA, but at the expense of fish growth (Chapter 6). VO best suited as a substitute for FO should contain high SFA and MUFA as energy sources and low amounts of LA because this fatty acid is poorly oxidized and is also difficult to remove from fish flesh using finishing diets (Turchini et al., 2009). Generally, compared to feeding on FO, VO will maintain a high growth rate for barramundi, but not necessarily a premium quality in terms of n-3 LC-PUFA content or the n-3:n-6 ratio, including when fed under sub-optimal conditions or immunity stress.

Plants such as Patterson's curse, *Echium plantagineum*, winter speedwell, *Veronica persica*, and blackcurrant, *Ribes nigrum*, have a Δ6 desaturase gene that produces the n-3 LC-PUFA precursor SDA (18:4n-3) and n-6 LC-PUFA precursor GLA (18:3n-6) from ALA (18:3n-3) and LA (18:2n-6), respectively, in their seeds. Therefore, Echium oil, from Patterson's curse, has SDA and GLA levels at >10% and 12%, respectively, depending on the locality and

strain, and only contains moderate levels of LA compared to other VO (Guil-Guerrero et al., 2001; Guil-Guerrero, 2007). EO has been investigated as an alternative to dietary FO in both marine and freshwater fish (Chapter 1). SDA was shown to be readily elongated to ETA (20:4n-3) in fish cells *in vivo* (Ghioni et al., 2002), suggesting that SDA-rich oils could be used in aquafeed formulations. As it is rich in SDA and GLA (18:3n-6), EO has the potential of increasing ETA and DGLA (20:3n-6) in fish tissues. Graded replacement of 80-100% FO with EO in Arctic charr, *Salveinus alpinus*, and Atlantic cod, *Gadus morhua*, diets did not affect growth performance, feed efficiency, survival and lipid content (Tocher et al., 2006a). Most importantly C_{18} PUFA in EO, such as ALA, SDA, LA and GLA, all increased in fish flesh and liver, while EPA and DHA were reduced (Bell et al., 2006; Tocher et al., 2006a).

Research on Atlantic salmon examined dietary EO as a complete replacement for FO. An initial trial with salmon parr showed that the contents of EPA and DHA in red and white muscle achieved by feeding EO were comparable to those obtained with feeding on FO (Miller et al., 2008b). In subsequent trials, the level of n-3 LC-PUFA was reduced in the whole body and separate tissues of salmon parr or smolts, or even through a prolonged feeding period covering these two phases (Codabaccus et al., 2011a; Codabaccus et al., 2011b). A blend of RO and EO (1:1) fed to salmon parr for 42 days also sustained the level of DHA in fish muscle when compared to the FO treatment with the author's suggesting that there could be a net DHA synthesis (Miller et al., 2007). A blend of FO:EO (1:1) was fed to gilthead sea bream, *Sparus aurata*, for seven months and the percentage of DHA in muscle and liver was unaffected, although there was a decrease in the absolute amount of DHA (mg/g dry weight) (Díaz-López et al., 2009). After 4 months of feeding the same blend to gilthead sea bream, their cell viability, total lipid content and lipid class composition in enterocytes and hepatocytes were not affected by EO. The cells clearly reflected the fatty acid profile of the EO showing increased SDA, GLA and its elongation product DGLA, with only a small decrease in n-3 LC-PUFA compared to the larger decrease occurring using other VO. The metabolism of [1-^{14}C]LA and [1-^{14}C]ALA was also unaffected by EO in terms of total uptake, incorporation, β-oxidation and elongation–desaturation activities (Díaz-López et al., 2010).

In this thesis, growth performance of 5 g size juvenile barramundi was reduced after eight weeks of feeding on EO compared with FO and rapeseed oil (RO). The n-3:n-6 ratio was higher in EO fish whole body (Chapter 2), skeletal muscle and liver (Chapter 3) compared with when feeding with the other VO. In subsequent trials with smaller (0.6 g, Chapter 6) or larger (50 g, Chapter 4 and 5) fish, EO was as efficient as FO in increasing fish weight. In all

feeding trials, EO failed to increase n-3 LC-PUFA in barramundi to comparable levels as achieved with feeding on FO. From a nutritional perspective, EO is not a suitable alternative to FO for complete replacement of FO in barramundi aquafeeds. Nonetheless, based on fish n-3:n-6 composition, EO has greater potential than other VO, when partially blended with FO in aquafeeds. These results suggest some promise for the use of naturally available SDA-rich oils in aquafeeds. The current low production volumes of EO mean it is prohibitively expensive as a practical alternative to FO in aquaculture. Such promise may be reached when the production of EO, or more likely other SDA-rich oils, expands and prices become competitive with other VO. Notwithstanding, the high n-3 LC-PUFA content in farmed seafood products achieved with FO diets will not be attained.

Most terrestrial VO are rich in LA and oleic acid (OA, 18:1n-9). In contrast, LO can contain over 50% ALA, but LO is not produced in comparable amounts to other VO (White, 2008; Gunstone, 2010). LO was fed for two weeks to juvenile barramundi (0.6 g) in comparison to EO or FO while the fishmeal used as the protein source was defatted (Chapter 6). Growth performance was reduced due to complete replacement of FO with LO, but not with EO. Furthermore, both LO - rich in ALA - and EO - rich in SDA - did not elevate the whole body content of n-3 LC-PUFA; both VO were less efficient in elevating LC-PUFA concentrations in barramundi compared to feeding on FO. In older juvenile (19 g) barramundi, six weeks of feeding with graded substitution of FO showed that 75% LO did not change the growth performance (Raso and Anderson, 2003), although residual n-3 LC-PUFA in the fishmeal may have compensated to achieve comparable growth rates. The total lipid content in barramundi carcass was not affected by the replacement level, while details on the fatty acid composition were not reported. In the diadromous Atlantic salmon, feeding 100% LO to parr in freshwater did not affect the growth or weight gain (Tocher et al., 2000). The authors suggested that feeding parr on LO prevented dietary inhibition of the desaturase activities, whilst smolts did not achieve comparable growth rates and LC-PUFA composition.

Feeding Atlantic salmon graded LO levels up to a complete replacement of FO did not affect growth or flesh lipid in extended (40 weeks) (Bell et al., 2004) and shorter (12 weeks) (Menoyo et al., 2005) experiments. In these studies, the graded decrease in dietary FO was reflected by graded decreases in EPA, DHA and arachidonic acid (ARA, 20:4n-6). The effects on tissue fatty acid compositions were quantitatively greater in the neutral lipid fraction than in the polar lipid. However, being rich in ALA, dietary LO increased the appearance of SDA and ETA (20:4n-3) in salmon liver and muscle. In a further trial with another salmonid,

complete replacement of FO with LO in the diet of rainbow trout, *Oncorhynchus mykiss*, for 72 days did not show any significant effects on fish growth performance (Turchini and Francis, 2009). Despite 8.8% of the net ALA dietary intake being bioconverted to n-3 LC-PUFA, the levels of EPA and DHA were significantly reduced when compared to feeding on FO. Marine fish such as gilthead sea bream and European sea bass were fed 60% LO for 14 and 34 weeks, respectively, without affecting their growth and final weights compared to fish fed FO (Mourente and Dick, 2002; Izquierdo et al., 2003; Mourente et al., 2005). These levels of dietary LO resulted in the percentage of ALA and LA increasing, while the proportion of EPA, DHA and ARA decreased in the total lipid of flesh, liver and blood cells in these fish. Similarly, in sharpsnout sea bream, *Diplodus puntazzo*, 13 weeks of feeding on 100% LO did not cause negative effects on fish growth and final weight. Flesh fatty acid composition had more GLA and reduced n-3 LC-PUFA (Piedecausa et al., 2007).

This array of reports on carnivorous fish species from different ecosystems suggests that the growth of fish fed on VO rich in ALA can be comparable to FO treatments if small amount of n-3 LC-PUFA are present in the fishmeal. However, regardless of EFA requirements, growth performance and compositional changes in freshwater, marine and diadromous fish fed LO, particularly at 100% replacement and including barramundi (Chapter 6), provided clear evidence that ALA cannot substitute n-3 LC-PUFA in achieving high growth rates. The efficiency of the endogenous conversion of ALA to EPA or DHA is very low and not necessarily restricted by the initial FAD6 alone. As marine and diadromous species require preformed LC-PUFA for better growth, VO rich in ALA are not considered as suitable complete replacement alternatives to FO. This thesis also demonstrated that neither ALA nor SDA-rich oils can increase n-3 LC-PUFA in either the whole body or tissues in barramundi at different sizes as the initial FAD6 is not the only obstacle in the biosynthesis pathway.

The global farming of the canola (CANadian Oil, Low erucic Acid) cultivar of rapeseed has increased since the early 1970s (Gunstone, 2004). RO is rich in monounsaturated fatty acid (MUFA), while LA and ALA dominate its PUFA composition, and has been used widely in aquafeeds with mostly efficient growth results. An abundance of growth trials have been implemented in the last three decades to evaluate the possible effects of FO replacement with RO for fish from different ecosystems and collectively have shown comparable growth performance (Turchini and Mailer, 2010). Few examples with relatively long growing periods reported any negative effect. One study reported that RO can replace FO in juvenile (19 g) barramundi diet up to 75% without encountering negative implications on growth

performance or body lipid content (Raso and Anderson, 2003). In Chapters 2 and 3, complete replacement of FO with RO in smaller (5 g) barramundi was successful in relation to growth and survival, even with the use of a defatted fishmeal. However, when bigger (50 g) fish were fed RO as a complete substitute for FO (Chapters 4 and 5), growth performance parameters were significantly reduced. The content of n-3 PUFA in barramundi fed RO was significantly reduced regardless of the starting size and growth efficiency. Fish species have specific essential fatty acid (EFA) requirements determined by their environmental and evolutionary history. Dietary LA and ALA satisfy the EFA requirements of freshwater fish or those from a low-trophic level (Torstensen and Tocher, 2010). In comparison, marine species as well as those from a high trophic level require a direct source of n-3 LC-PUFA (Tocher, 2003). When VO such as RO, rich in MUFA compared to PUFA, are used in aquafeed, an additional source of preformed EFA is still required to assist in efficient growth. These requirements are normally met by the n-3 LC-PUFA contained in fish meal or by the inclusion of small amounts of FO (Turchini and Mailer, 2010). A relatively low n-3:n-6 ratio in the whole body and tissues resulted from feeding barramundi on RO compared to FO, EO or LO (Chapters 2, 3, 4, 5 and 6). This suggests that MUFA-rich and PUFA-poor VO are not suitable lipid sources for aquafeeds to produce a premium quality fish, in terms of n-3 LC-PUFA content, for the final consumer, although the growth rates may be comparable at certain stages of the grow-out phase.

Other VO, such as SO, have been fed to barramundi as partial or complete replacements to FO. Recorded growth parameters varied according to the level of inclusion, but less information is available on changes in FA composition (Catacutan and Coloso, 1997; Raso and Anderson, 2003). Information on the use of alternatives to FO other than VO in barramundi aquafeed has not been documented. Single-cell oils or biomass, rendered animal fat or other lipid sources tested on other commercial fish may provide promising alternatives to FO for use in barramundi aquafeed.

7.3. Biosynthesis of n-3 LC-PUFA: regulation and capacity limits

This thesis has demonstrated, through indirect quantification of enzyme activity in the whole-body by the combined use of (i) FA mass balance calculations, (ii) measurement of tissue-

specific FA composition and (iii) quantitative gene expression, that barramundi has an endogenous capacity to synthesize n-3 LC-PUFA from C_{18} PUFA precursors, albeit to a limited extent (Chapter 2 and 3). The bioconversion of precursors into neutral lipid LC-PUFA appears to be limited by factors not only restricted to the initial FAD6 step. It was also shown that high concentrations of n-3 LC-PUFA from dietary FO are accumulating in the fish body to redundant levels and deplete rapidly under sub-optimal conditions (Chapter 4 and 5). This finding suggests that the observed limited endogenous capacity for biosynthesis of n-3 LC-PUFA from their C_{18} precursors in barramundi could be the minimal required levels. High levels of dietary SDA and GLA enhanced n-3 LC-PUFA biosynthesis by bypassing the rate-limiting desaturation step. However, in comparison to the FO treatment, complete replacement of FO with EO rich in SDA and GLA reduced the levels of LC-PUFA in barramundi muscle, liver and the whole body. LC-PUFA biosynthesis pathways are complex and controlled by a set of biochemical factors and rate-limiting steps other than the initial FAD6 rate-limiting step.

Despite requiring FAD5 activity in LC-PUFA biosynthesis, molecular and chemical studies did not detect significant activity for this enzyme in barramundi (Mohd-Yusof et al., 2010), which can be considered as another rate-limiting step in the LC-PUFA biosynthesis pathway. While homologous expression of barramundi FAD6 in yeast showed that only 32% of ALA is converted to SDA, conversion rates of ALA to SDA in barramundi hepatocytes *in vitro* is higher than the rate known for Atlantic cod, *Gadus morhua*, but lower than that for Atlantic salmon (Tocher et al., 2006b; Mohd-Yusof et al., 2010). FA compositional changes and the indirect *in vivo* quantification of LC-PUFA biosynthesis in tissues and the whole body reported in this thesis complement previous *in vitro* biochemical work and further validate the limited capacity of barramundi to convert ALA or SDA to EPA and DHA. It is also shown that there are limitations in the n-6 LC-PUFA biosynthesis pathway given the low net production of ARA from dietary LA and GLA in barramundi. To have n-3 or n-6 C_{18} precursors for LC-PUFA must compete for existing enzymes shared between the two biosynthesis pathways through the parallel reactions (Tu et al., 2010; Hofacer et al., 2011). Therefore, it is plausible that n-6 LC-PUFA biosynthesis from their precursors in barramundi is not efficient when n-3 LC-PUFA biosynthesis is also limited as shown in Chapter 2 and 3 by different approaches.

Results presented in Chapters 4 and 5 showed a reduction in n-3 LC-PUFA to a low and comparable concentration in stressed (with reduced feed intakes) (3-7 days) fish fed previously on FO, EO and RO. Compositional estimation concluded that LC-PUFA retention decreases with increasing LC-PUFA inclusion in barramundi diet, and dietary DHA as low as 1% is required for optimal growth and healthy fish (Glencross and Rutherford, 2011). However, the minimum requirements of barramundi for total n-3 LC-PUFA under optimal and sub-optimal conditions remain to be established empirically, from the gene to the whole organism level. Similarly, how efficient barramundi are in remodelling their lipid or selectively retaining LC-PUFA to keep the minimal and vital LC-PUFA concentration also remains to be determined.

Barramundi fed on EO had higher concentrations of C_{20} LC-PUFA, such as EPA and ARA, in the polar lipid compared to the neutral lipid fraction (Chapter 3). The FA profiles were influenced mainly by dietary oil rather than the environment; changes may be explained either by selective retention or accumulation of final products synthesized from dietary precursors into polar lipid. With feeding on EO, an alternate mechanism probably lead to selective retention of EPA and ARA in the phospholipid fraction as a consequence of normal lipid remodelling and turnover. This thesis provides an overview on barramundi lipid nutrition and its interaction with selected environmental conditions under experimental settings. However, it is still not fully understood how dietary and environmental factors can modulate genes and enzymes regulating barramundi FA metabolism under more complex factorial designs or in fish farms. The magnitude of response in gene expression in these pathways is also dependent on the genome of the fish and the target tissue when FO is replaced by VO (Panserat et al., 2009; Leaver et al., 2011; Morais et al., 2011). Comparative studies of gene function and distribution integrated with recent fish genome sequence and breeding programs provided insights into lipid metabolism and the outcomes associated with the replacement of FO in fish diets (Leaver et al., 2008; Bell et al., 2010). Through combined use of genetic markers, transcriptomic, proteomic and biochemical analyses, it may be possible in the future to identify barramundi families and strains better adapted to alternative diet formulations and diet-environment interactions.

7.3.1. ABIOTIC EFFECTS OF AMBIENT CONDITIONS

Unlike terrestrial animals, the fish body is immersed in water and their bodily fluid compartments and tissue systems are in direct or indirect contact with ambient water (Randall et al., 2002; Cossins and Crawford, 2005). Euryhaline and eurythermal teleost species, such as barramundi, have the ability to live in different environmental conditions and be exposed to a range of changes in their natural habitats (Chapter 1). Changes in salinity and temperature are key environmental factors in fish lipid metabolism through their influence on unsaturation levels, homeoviscous adaptation and remodelling of lipid class composition to compensate for salinity and temperature changes (Käkelä et al., 2008; Hölttä-Vuori et al., 2010).

Temperature affects both fish metabolism and feed conversion, which may influence the turnover rate of FA within fish muscle, liver and adipose tissue. The interaction of temperature and diet can cause FA to reflect the diet in a non-conservative manner (Elsdon, 2010). Changes in dietary FA significantly control the regulation of gene expression and lipid biosynthesis and FA composition (Chapter 2, 3 and 4). Besides the dietary factors, environmental changes also play a major role in regulating fatty acid composition in barramundi tissues (Chapter 4).

Barramundi tolerate a wide range of salinity and perform well in hypersaline water of up to 40 ppt (Partridge and Lymbery, 2008; Salama, 2008). Results reported in Chapter 2 and 3 showed that growth efficiency and lipid metabolism of this euryhaline fish were not affected by water salinity and provided deeper insight into the lipid metabolism. The distinctive ability of barramundi to adapt to different salinities without having significant changes to lipid metabolism or composition has not been recorded previously for other diadromous (Codabaccus et al., 2011b; Mizuno et al., 2011) or euryhaline fish (Borlongan and Benitez, 1992; Li et al., 2008; Xu et al., 2010).

Before achieving the current level of understanding for nutritional biochemistry and genetics, most of the emphasis in terms of evaluating nutritional physiology was placed on examination of effects of abiotic environmental factors. With recent advances in nutritional biochemistry, the study of diet-environment interactions is now performed in physiological studies at the molecular and biochemical level as a mechanistic approach in nutrition studies (Quinton et al., 2007; Ordovás et al., 2011). A growing body of evidence supports the notion of environment-

diet interaction, which regulates the expression of genes involved in lipid metabolism and many other metabolic processes (Kolditz et al., 2010; Rideout et al., 2010; Smith, 2011). Barramundi fed on VO as a complete replacement for FO had comparable growth rates but reduced n-3 LC-PUFA content under optimal or sub-optimal environmental conditions. In this thesis, combining basic molecular applications with a range of analytical chemistry, mathematical and immunoassay quantification of enzymatic activity enabled the alignment of metabolism dynamics with compositional changes in fatty acids. Establishing an enhanced understanding for lipid metabolism pathways in fish, and the feedback mechanisms controlling these reactions, is one of the overarching aims of aquaculture nutrition research. Most barramundi farms, and those for many other fish species, are outdoors and exposed to environmental changes on a daily or seasonal basis. Continuing research on the interactions between diet and environment and their effect on fish growth, quality and physiology will improve aquaculture production and profitability.

7.3.2. IMMUNITY STRESS

Systematic evaluation of diet-gene-environment interactions in boosting immunity and reducing disease risk has been developed recently and replaced the nature *vs*. nurture controversy (Hernandez and Blazer, 2006; Traynor and Singleton, 2010). The relationship between nutrition and infections are synergistic, with the combination of the two being greater than could be predicted from either alone (Scrimshaw, 2007). The effect of dietary fats on the immune system of fish depends not only on the species studied and the essential fatty acid requirements of that species, but also on the levels of other nutrients related to lipid metabolism (Waagbø et al., 2003; Puangkaew et al., 2004).

The results from a range of studies where FO was replaced partially or completely by different vegetable oils indicated that the source of vegetable oil did affect fish immune response. Dietary VO caused certain changes in fish immunity such as reduction in macrophage respiratory burst activity in European sea bass, *Dicentrarchus labrax* (Mourente et al., 2005), and gilthead sea bream, *Sparus aurata* (Montero et al., 2003). Resistance of erythrocytes to hemolysis in hypotonic solutions increased with increasing level of dietary n-3 LC-PUFA in channel catfish, *Ictalurus punctatus*. However, immunosuppressive effects such

as lysozyme activity and serum complement activity were also associated with excessive levels of FO (Yildirim-Aksoy et al., 2009). A general trend was also observed in marine and diadromous fish fed decreased content of LC-PUFA; the production of eicosanoid metabolites, derived from either EPA or ARA, was reduced. Partial or complete replacement of FO with VO significantly lowered the concentration of plasma prostaglandin E_2 in European sea bass (Mourente et al., 2007) and Atlantic salmon (Petropoulos et al., 2009). Dietary VO rich in SDA inconsistently affected eicosanoid metabolism and production in marine fish (Bell et al., 2006; Villalta et al., 2008).

In Chapter 5, dietary EO rich in two FA beyond the initial FAD6 step, SDA and GLA, significantly improved the immune response, in terms of eicosanoid production, in the diadromous barramundi compared to RO lacking these two FA. However, compared to VO, FO was significantly efficient in enabling the fish to overcome immunity stress. These results were supported by a distinct reduction of key eicosanoid metabolites involved in the inflammatory response to bacterial infection in blood plasma of fish fed EO compared to RO. The findings concur with previous research on other species and highlight the important role of diet, in particular the n-3 PUFA and n-3 LC-PUFA, in modulating immune response and disease resistance in barramundi (Chapter 5).

7.3.3. ONTOGENETIC EFFECTS

Quantitative and qualitative requirements for EFA and the n-3:n-6 ratio vary ontogenetically in fish with early developmental stages and broodstock being critical periods (Glencross and Turchini, 2010; Tocher, 2010). Estimated EFA requirements for juvenile and sub-adult freshwater and diadromous fish studied so far indicate that they can be satisfied by the C18 PUFA, ALA and/or LA, at around 1% of the diet dry weight, in contrast to marine species which require preformed LC-PUFA (Tocher, 2010). Results reported in this thesis have clearly demonstrated that the capacity of barramundi to endogenously synthesise LC-PUFA from their C18 precursors supplied though the use of dietary EO is relatively limited regardless of the dietary FA composition and ambient conditions (Mohd-Yusof et al., 2010) (Chapter 2, 3, 4 and 5). Competitive affinity between desaturases, elongases and other FA metabolism enzymes to act on n-3 or n-6 PUFA also limits the endogenous conversion of

precursors to LC-PUFA in these two FA series. Therefore, changes in the qualitative composition of PUFA in barramundi diets may be reflected firstly on their growth performance. These requirements may vary as a function of age in euryhaline species experiencing changes in natural conditions as a part of their life cycle, but no clear evidence is available on such interaction.

In the experiments performed in this thesis, a variety of initial sizes of juvenile barramundi were fed on diets with different n-3:n-6 ratios. The estimated specific growth rate (SGR, % day^{-1}) from the experiments in which the average initial fish size was 0.6 g (Chapter 6), 5 g (Chapter 2 and Chapter 3), 50 g (Chapter 4 and Chapter 5) and 176 g (Williams et al., 2006) were plotted against n-3:n-6 ratio in a multiple linear regression (PASW 18.0, SPSS Inc., USA) (Figure 7.1). All SGR values were calculated for 8 weeks of growth in this analysis. When the experiment lasted less than this period, SGR values were adjusted by predicting final weight from actual growth rates (Alanärä et al., 2001) then recalculating SGR after 8 weeks. Normalising experiment duration equalised the time-line for SGR comparisons at an endpoint when fish biomass at least doubled the starting size to reflect the diet effects on their chemical composition. The duration for aquaculture experiments is fixed in relation to biological scale, while the body composition of fish show temporal changes influenced by factors such as diet composition. Thus, for a growth experiment it is advised to aim for increasing biomass 2-3 fold during the trial (Shearer, 1994; Guillaume, 2001). Only fish raised at 29-30°C were considered for this analysis regardless of the experimental salinity. SGR was negatively correlated with weight (r^2 = -0.869; P = 0.002; n = 12), but not with the n-3:n-6 ratio (r^2 = 0.275 P = 0.193, n = 12). Barramundi size did not correlate with n-3:n-6 ratio (r^2 = 0.331; P = 0.148; n = 12). Linear regression, where fish weight and dietary ratio of n-3:n-6 are the predictors and SGR is the dependent variable, was significant (r^2 = 0.755, P = 0.002, n = 12). The multiple regression model is described by the following equation:

$$SGR = 4.686 – 0.017 (weight) – 0.014 (n\text{-}3:n\text{-}6)$$

Biological interpretation of the quantified regression indicates that barramundi growth is fastest at smaller sizes when dietary n-3 PUFA is at double the level of n-6 PUFA. When the n-3:n-6 ratio exceeded 2, the SGR decreased slowly in juveniles larger than 60 g. These

observations indicate the interactive effect of fish size and their diet quality, in terms of PUFA composition, on the growth rates. Increasing the level of FO to 30% in the diet of juvenile (55 g) barramundi reduced their growth compared with a commercial diet containing 12% lipid from an undisclosed source (Raso, 2004). Therefore, increasing n-3 LC-PUFA in barramundi aquafeed beyond optimal levels is not only inefficient practice, but also appears to reduce SGR. One data point (at 176 g) was extracted from a study where the diet formulation was not identical to those used in this thesis and the protein was provided as a mixture of fishmeal and casein. As noted in section 7.2, different diet components and compositions may influence barramundi growth differently and can, therefore, explain the slight drop in the slope at the point where some experimental materials were not similar to those from other data-points on the same slope.

Low, optimal, and high n-3:n-6 ratio in the diet seems to affect the growth of farmed fish in different patterns. In a graded replacement study replacing the LA-rich soybean oil with ALA-rich linseed oil (and changing the n-3:n-6 ratio) in Tench, *Tinca tinca*, growth performance was lowest in the fish receiving the lowest ALA:LA dietary ratio (Turchini et al., 2007). The final weight and weight gain of hybrid tilapia (female Nile tilapia *Oreochromis niloticus* × male blue tilapia *O. aureus*) was also higher when n-3:n-6 ratio ranged between 0.6 and 2.1, while growth was reduced at a ratio of either 11 or lower than 0.1 (Chou and Shiau, 1999). The growth of Murray cod (Francis et al., 2009; Turchini et al., 2010), Eurasian perch (Blanchard et al., 2008) and sharpsnout sea bream, *Diplodus puntazzo* (Piedecausa et al., 2007) was less responsive to changes in dietary n-3:n-6 PUFA ratios. These differences can be attributed to the levels of SFA and MUFA in the diet which interfere with PUFA absorption and digestibility and affect fish growth (Olsen and Ringø, 1997; Turchini et al., 2009). Overall, barramundi SGR is highest when dietary n-3:n-6 ratio is between 2 to 4 and reduced at lower or higher ratio values (Figure 7.1).

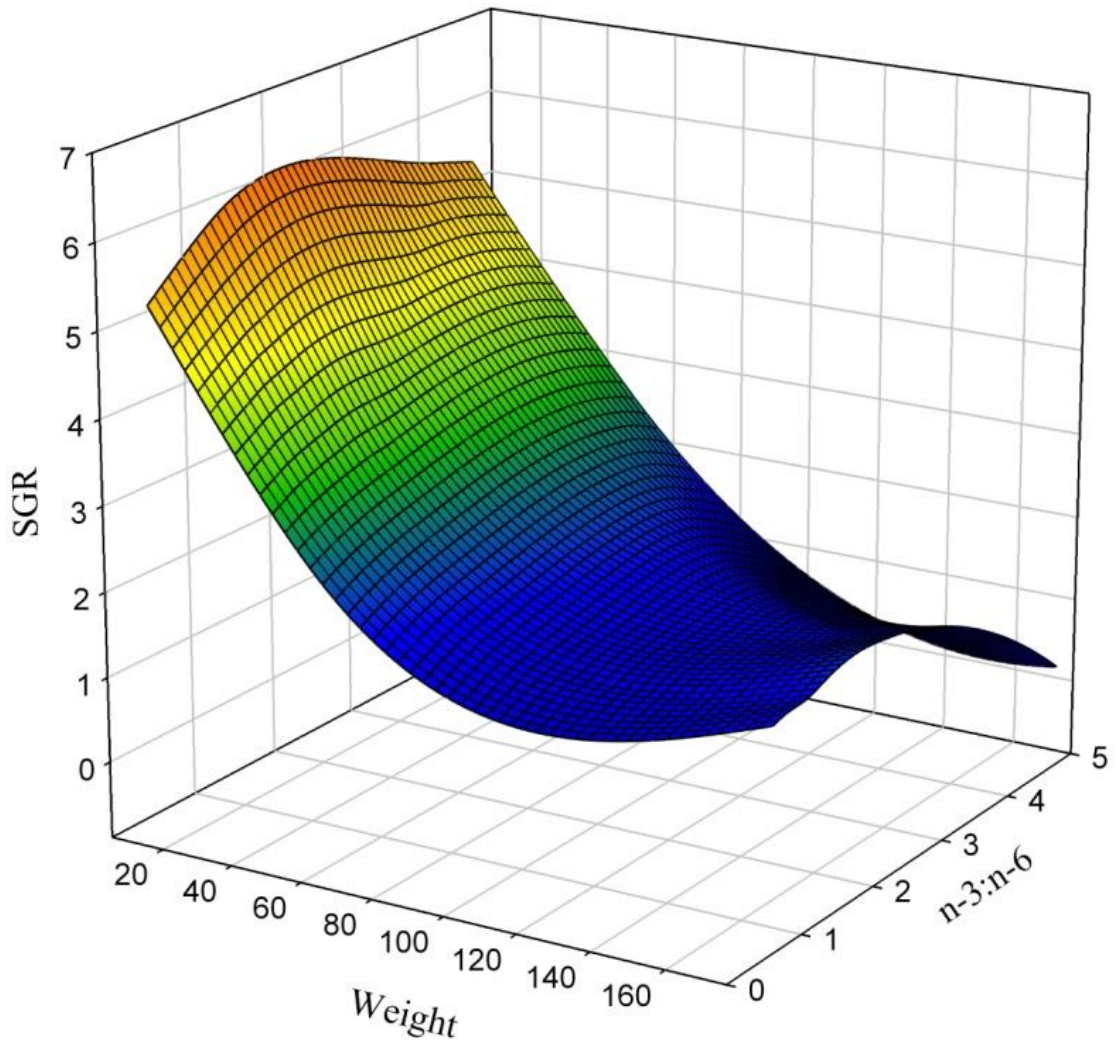

Figure 7.1: Relationships between initial weight (g), dietary n-3:n-6 ratio and SGR (% day^{-1}) for barramundi calculated through 8 weeks (Section 7.3.3). Data source: initial weight 0.6 g (Chapter 6), 5 g (Chapter 2 and 3), 50 g (Chapter 4 and 5) and 176 g (Williams et al., 2006).

7.3.4. MODULATION OF LC-PUFA BIOSYNTHESIS BY BIOACTIVE COMPOUNDS

Dietary inclusion of bioactive compounds such as flavonoids (Morin et al., 2008; Nichols et al., 2011), sesamin (Lim et al., 2007; Ide et al., 2009), phytosterols (Brufau et al., 2008; Xu et al., 2008) and conjugated linoleic acid (Diez et al., 2007; Corl et al., 2008; Murphy et al.,

2009) provided additional evidence of gene-nutrient interactions in a range of fish and other species. Previous *in vitro* and *in vivo* studies have claimed various metabolic effects of these compounds, mainly in modulating lipid metabolism (Diez et al., 2007; Lim et al., 2007; Nichols et al., 2011). Understanding the effect of these compounds has been examined previously with mammals (Hirose et al., 1991; Fujiyama-Fujiwara et al., 1992; Ashakumary et al., 1999) and recently tested on commercial fish of different sizes, from differing ecosystems and with varying dietary habits (Trattner et al., 2008; Mráz et al., 2010; Trattner et al., 2010). Sesamin was found in separate studies to affect expression of genes and enzyme activity involved in lipid metabolism, mainly LC-PUFA biosynthesis. In Chapter 6, the effect of dietary sesamin on barramundi in their early life stage was tested in terms of changes in lipid class, fatty acid and sterol composition in the whole body. As a result of the modulation of lipid metabolism by sesamin, total lipid content was reduced and growth performance was compromised. However, levels of n-3 LC-PUFA in the whole body and the conversion of dietary LC-PUFA precursors to final products were significantly higher in fish fed EO in combination with sesamin. This observation suggested that sesamin may have potential for increasing the rates of n-3 LC-PUFA biosynthesis in barramundi fed on VO, although, rather than as juveniles, at more advanced life stages. Further research will be needed to follow up on this interesting initial finding with barramundi.

7.4. Conclusions and recommendations

- SDA-rich oils have a potential to replace FO in barramundi aquafeed, partially or completely, and will result in fish rich in n-3 PUFA and n-3 LC-PUFA which the resulting seafood being highly suitable for human consumption.
- Barramundi has malleable trends of lipid metabolism and growth rates under biotic and abiotic stress, which make it an excellent species for the expansion of aquaculture outdoor farms.
- Plant-derived bioactive compounds can be used as dietary additives to enhancing the quality of barramundi fed on VO and to reduce the reliance on FO in aquaculture.

7.5. Prospective of FO alternatives in barramundi aquaculture

Limits to the harvest from wild fisheries are becoming increasingly evident and the expanding aquafeed industry is also placing greater demand on available marine resources such as fish oil derived from forage fisheries (Tacon and Metian, 2009; Péron et al., 2010; Smith et al., 2011). It is clear that aquaculture will not be able to secure substantially greater volumes of fish oil by utilizing the lower trophic level forage fish from the oceans (Alder et al., 2008; Tacon and Metian, 2008; Naylor et al., 2009). A number of alternative sources for n-3 LC-PUFA can be considered to counter these increasing constraints on the previously traditional sources of fish meal and in particular fish oil. Some terrestrial vegetable oils appear to provide practical alternatives to FO in barramundi aquafeeds without compromising fish growth rates and their ability to cope with environmental stressors. However, no naturally-available VO can replace FO in barramundi feeds and maintain high n-3 LC-PUFA content in fish products. A mixture of VO and other oils can contain n-3 LC-PUFA from renewable, underutilized or emerging sources to supply ingredients for a responsible and sustainable aquaculture industry. Better use of fisheries by-products and seafood processing waste from the existing harvest can produce increased amounts of n-3 LC-PUFA. Other promising opportunities exist with single cell organisms and in particular genetically-modified terrestrial plants that can produce n-3 LC-PUFA (Miller et al., 2008a; Turchini et al., 2012). Further research on stimulating the metabolic pathways of LC-PUFA biosynthesis may also assist in triggering the dormant capacity of farmed fish to make n-3 LC-PUFA from sustainable and renewable resources.

7.6. References

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